

- I. A disease of Norway spruce (Picea excelsa (Lam.) Link.) associated with Stereum sanguinolentum (A. et S.) Fr. and Pleurotus mitis (Pers.) Fr.
- II. Studies on the biology of Auricularia auricula-Judae (Linn.) Schroet. (= Hirneola auricula-Judae (Linn.) Berk.) causing rot in elder (Sambucus nigra L.)
- III. An oak (Quercus robur Linn.) canker caused by Stereum rugosum (Pers.) Fr.

by

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## INTRODUCTION.

It has been observed that about 20 per cent of forty to sixty years old Norway spruce trees (Picea excelsa (Lam.) Link) growing in a dense, mixed stand with Sitka spruce and Douglas fir at Glentress forest near Peebles in Scotland, have been for the last four or five years in a diseased condition and are gradually dying. The trees are badly shaped and in most cases there is a considerable amount of resin flow from the bark on the lower trunk. The trees do not give any evidence of the presence of frost rings or cracks in their wood. This plantation occupies a hill-slope of moderate gradient, leading to the top of the hill (about 700-1000 feet above sea-level). On the whole it is a cool, damp and muddy situation and areas of bad drainage occur here and there. Since the economic value of Norway spruce as a timber-yielding plant is well known, it is thought that the information concerning the causal agent and the amount of damage caused by it is desirable. With this end in view, the present investigation has been undertaken during the latter half of 1949. It is interesting to note here that during this investigation two fungi, viz., Stereum sanguinolentum and Pleurotus mitis have been found to be associated with the disease and

these are capable of infecting living trees through wounds, eventually causing rot in the wood. It is apparent that this association of two pathogens has greatly enhanced the importance and complex nature of the problem. At the present moment it is difficult to estimate the relative importance of the two fungi in bringing about the decay in living trees and the nature of reactions existing between them. An investigation of this nature would necessarily require a longer period owing to the slow growth of the fungi and their effects on a living host. The data, so far obtained, have been given in the following pages based largely on studies in the laboratory as well as in the field.

Gaumann and Jaag (1937) have described a disease in spruce and fir causing severe damage in a re-forestation area of the Emmentel in Switzerland. The symptoms described by them are somewhat similar to those observed in the present study and are attributed only to the virulent pathogen, Pleurotus mitis. Although this fungus has been known to cause heart-rot of conifers on the continent of Europe, yet up to the present it does not appear to have been recorded on living trees in Great Britain.

Recently, Day (1950) has reported a severe case of bark necrosis on Norway spruce in Drummond Hill Forest in Perthshire, Scotland. The injuries are diagnosed by him as having been caused by frost



damage usually after pruning live branches. He has reported and figured cracking of the bark with consequent outflow of resin. The effect of the injuries on the wood of the tree, however, has not been fully investigated by him. He mentions that under such conditions some degree of fungal infection is inevitable although it has not yet been possible for him to say what types of fungi may have entered.

The same type of injury has been reported by Laing (1947) on Sitka spruce and the cause of such damage has been attributed to Nectria cucurbitula. He has found abundant mycelium and conidia of Nectria cucurbitula on the dead bark but he did neither actually isolate the fungus from the internal tissues nor carried out any infection experiments to prove its pathogenicity. His statements on pathogenicity are entirely based on those given by Hartig.

Although Stereum sanguinolentum has long been known to occur commonly on dead stumps and branches of coniferous trees in Great Britain and on the continents of Europe and America, there still exist contradictory statements in the description of the rot made by various workers from time to time. As such it will be evident that the external symptoms exhibited by the infected trees under consideration are somewhat different from those described by previous workers. In Great Britain very little is known about its parasitic activity although it is



reported to cause a butt-rot of coniferous trees (Day and Peace, 1936) and timber-stain in Norway spruce (Cartwright 1937). Cartwright and Findlay (1946) have isolated it from freshly felled poles of spruce and larch and incidentally referred to its sporadic occurrence in living trees. In the continent of Europe it occurs on various conifers in Denmark (Ferdinandson and Jorgensen, 1938-39) and Germany (Bergenthal, 1933) and also as a wound-parasite in fir and pine (Rohmeder, 1937). In Russia, it causes a slow decay in fir and pine (Vakine, 1934). This fungus is also known to attack living spruce in Norway (Jorstad and Juul, 1939) and in Sweden (Lagerberg, 1919, 1923) where it gains entrance through top-breakage due to snow damage or through wounds. Robak (1936) has isolated it from heart-rot of living spruce and mentioned (Robak, 1942) that it is commonly found on dead coniferous wood in Sweden. In America, it causes a heart-rot of balsam fir (Faull and Mounce, 1924; McCallum, 1925, 1928), often causing a top-rot (Kaufert, 1935). It also induces a sap-rot causing much damage in firs, Douglas firs, larches, pines and spruces (Hubert, 1935; Baxter 1937). It is a dangerous fungus in pruning wounds of northern white pine (Spaulding and Coll., 1935) and the principal agent causing 'slash' decay (Spaulding, 1929). Thus, it becomes evident that the importance of the fungus has been gradually recognised although it is

difficult at the present moment to estimate the amount of losses caused by this fungus but the indications are such that it may prove to be a serious pathogen in Great Britain and the losses caused by it may ultimately become very serious.

II. SYMPTOMS.

The symptoms of the disease are few and conspicuous. The infected trees show longitudinal depressions of the bark (Plate I fig. 1 ) which may often be ill-defined and somewhat flat. These extend from the butt upwards up to 6 feet, but occasionally up to a height of 10-12 feet, giving the trunk a somewhat fluted appearance. The bark of the sunken areas is often discoloured, its underlying tissues die and become dessicated and eventually it splits lengthwise accompanied by copious outflow of resin. (Pl. I Fig. 2 ) Gradually, these areas appear to sink deeply into the stem owing to continued growth in thickness in the areas lying between them. The lesions usually occur round about pruned branches, run up and down the stem and are restricted within the limits of pruning but may sometimes extend towards the base of the trunk where fluting becomes most pronounced. The bark near the base of the trunk ultimately becomes loose and separates easily, particularly when the poles are cut and dried. A mass of whitish discoloured tissue and mycelium is often present in between the bark and the wood. The inner surface of the bark and the surface of the wood in contact with it show a white mottled appearance.

Cross sections of freshly felled poles in the

early stages of decay show irregular to wedge-shaped areas (Pl. II figs. 5, 6 ) at the periphery which are light to dark brown, sometimes reddish-brown in colour. These gradually extend towards the centre of the wood into the base of the trunk and finally coalesce causing a butt rot. (Pl. II fig. 8 ). In such cases the course of infection appears to be from the bark to the centre of the stem. Close examination reveals that the cambium is killed near the pruned branches, possibly due to the entry of the pathogens through dead branches or other injuries such as pruning cuts and after killing the cambium they spread from the periphery to the centre of the wood. The organism seems to spread much more rapidly in a longitudinal direction than laterally. In this way, longitudinal strips of cambium are killed near the pruned branches and further secondary growth cannot continue. In the areas between these dead cambial strips growth continues and the stem ultimately shows prominent ridges. The transition from the normal to decayed wood is abrupt and there is no sign of any black line of decay. The discoloured areas, in an advanced stage, show small pockets which are somewhat whitish and hollow.

There was no sign of any fructification of the causal organisms on the surface of the infected trees but recently felled logs of such trees that had lain

on the forest floor were found with fructifications of Stereum sanguinolentum and Pleurotus mitis over the rotted areas on the surface of the cross sections (Pl. I figs. 3 ).



### III. THE CAUSAL AGENTS.

#### (a) Materials for Study.

The materials for study were obtained in November 1949, in the form of freshly felled infected trees from Glentress forest near Peebles in Scotland. The fructifications of Stereum sanguinolentum and Pleurotus mitis were also collected while growing in an excellent condition on the surface of the cross sections of recently felled Norway spruce poles lying on the floor of the forest near the infected trees. The trees studied in this connection were carefully selected, felled and macroscopically examined in the field to note, as far as possible, the progress of decay in the wood. For further study, small sections from different regions of the trunk were selected, brought into the laboratory, examined and studied while in a fresh condition. Microscopic examination of the rotted areas revealed the presence of abundant mycelium within the tissues of the host. Similar infected poles, collected in September, 1948, and kept in the laboratory, were also examined and studied.

#### (b) Isolation and Identification of the Causal Organisms.

The mycelium from the host tissues was isolated

and studied. The isolation was made from the infected wood by using portions of isolated rotted tissues from selected regions of the same or different poles. Small pieces (about 2" x  $\frac{1}{2}$ " x  $\frac{1}{2}$ ") of such wood blocks were washed several times with sterile distilled water, dipped quickly in absolute alcohol and flamed in order to free the surface, as far as possible, from superficial contaminations. One side of the exterior portion of such a wood block was removed with a sterile scalpel, a small piece of wood was then carefully cut out from the freshly exposed surface near the edge of the decayed area and transferred aseptically to a tube containing 2% malt agar slant into which it was partially embedded. In this way several transfers were made. Other transfers were also done from similar infected spruce poles collected in 1948 from the same plantation. Cultures were also made from poles bearing fructifications of Stereum sanguinolentum and Pleurotus mitis on their cross-section-ends. The inoculum in such cases was about a centimetre long, as viable mycelia were likely to be present in a large piece of inoculum than in a small one. In such cases it was necessary to soak the wood blocks first in distilled water for a few hours before the transfers were made. This was necessary in order to give the internal mycelium a start to grow from its

inactive condition within the dried wood. These were then incubated at 23°C. in darkness. In most cases the mycelia grew out within a week into the surrounding medium and sub-cultures were made from each of these in 2% malt agar tubes which were subsequently used as stock cultures. Growth of the mycelia from wood blocks obtained from spruce logs collected in 1948, was very slow and in some cases no growth appeared at all as the fungus might have gone into a resting condition or died. A few cultures were however, contaminated with mould and hence rejected. After close examinations of all the isolates it was subsequently found that these cultures belonged to two different fungi and exhibited the characteristics of the cultures of Stereum sanguinolentum and Pleurotus mitis. These were compared with the polysporous cultures of Stereum sanguinolentum and Pleurotus mitis already made available for the purpose and found to be identical with the latter in both cultural and microscopic details. The identity of these isolates was further confirmed when these cultures later on produced typical fruiting bodies in artificial culture (Plate. VII figs. 33-36).

Spore cultures of Stereum sanguinolentum and Pleurotus mitis were made in the following way. A

small portion of a fresh fruit-body of each fungus was fixed excentrically to the inner side of the upper lid of a Petri-dish containing 2% sterile agar and was placed in position with the hymenial surface downwards. These were then kept within a bell-jar lined inside with moist blotting paper. Within a few hours there was copious discharge of spores on the agar surface from which initial polysporous cultures were made by transferring the spores aseptically to malt agar tubes and were incubated at 23°C in darkness. The spores germinated readily on the surface of the agar plates within 12 to 24 hours. A good mycelial growth was obtained in all the tubes within a fortnight and these were kept as stock cultures for further study and comparison.

In a great majority of cases the isolates were those of Stereum sanguinolentum but nevertheless Pleurotus mitis was also isolated from separate regions of the same or different poles. In one instance both the organisms were not only isolated from the same pole but the respective rots were only a few centimetres apart from each other, being separated by healthy tissues. This statement was further corroborated by inducing the fungi to produce fructifications on the rotted areas at the cross-section-end of the infected pole from which isolations were made (Plate I fig. 4 ) From the



number of isolations so far made, it was evident that Stereum sanguinolentum was more widely distributed within the host-tissues than Pleurotus mitis. Further investigations in future with isolations made from large numbers of infected trees would possibly throw some light as to their relative distribution within the host plant.

(c) Descriptions of the Sporophores

After careful macroscopic and microscopic examinations of the sporophores of Stereum sanguinolentum and Pleurotus mitis an attempt has been made to give detailed descriptions in the following pages, based on materials which developed on sections of freshly felled infected poles of Norway spruce within a couple of months after they have been brought into the laboratory and kept outside in the open during the months of October to December. The microscopic structures were studied from free hand sections mostly cut from pieces of fresh sporophores. When dried specimens were used the usual procedure recommended by Burt (1914) and Overholts (1929) were followed and these proved satisfactory. In describing the hyphae, small groups of hyphae were teased from sections of different parts of the fruiting bodies, the sections being moistened first with a drop of 7% KOH solution. Overholts (1929) states that



sections cut and mounted in this way show the fungus-tissue in a remarkable state of turgidity comparable to that of fresh material. For temporary mounts the sections were stained with 1% aqueous eosin or lactophenol-cotton-blue with satisfactory results. Staining of the hyphae was found to be unnecessary, for the hyphae when mounted in lactophenol and the sub-stage diaphragm and the condenser slightly regulated, their visibility was decidedly increased. The colours used in the following descriptions are according to Ridgeway (1912).

1. STEREUM SANGUINOLENTUM (A. et. S.) Fr.

(Plate III figs. 11, 12)

Fructification - Sessile; at first forming somewhat orbicular and resupinate patches, then narrowly reflexed, later becoming laterally confluent and forming broad continuous sheet, measuring 30 - 40 x 30 - 50 m.m. or more; reflexed portions about 2 m.m. deep from margin to back, often becoming densely imbricated and laterally confluent, somewhat folded; dimension of the resupinate position usually 10 - 15 m.m. but in younger specimens much less (2-3x3-5 m.m.); thin and leathery; margin thin, acute, usually white in colour.

Upper surface - Villose; with somewhat satiny and radiately adpressed hairs; faintly zoned near the margin; pinkish buff or pale olive buff to pale drab gray or pale smoke gray towards the margin.

Context - Thin, coriaceous, whitish.

Hymenial surface - Smooth, often with small isolated tubercles; slightly wrinkled at places; becoming irregularly cracked in the resupinate portion on drying; bleeding red when cut or bruised; pale olive buff, olive buff or fuscous, becoming avellaneous or wood brown on drying.

Basidia - Cylindrical to clavate; dimension about 30-40 (50) x 4-8 $\mu$ ; di - to tetra-sterigmatic; sterigmata 4-5 $\mu$  long (*text-fig. I, a*).

Spores - White in mass, otherwise hyaline; smooth; oblong or slightly curved, with an apiculus; dimension about 6-7.5(8.5)x2-3 $\mu$ . (*text-fig. I, b*)

Cystidia - None.

Lactiferous cells - numerous; reddish-brown in colour; arising from the layer below the hymenium and extending into it; elongated but clavate near the apex; about 300-400x4-5 $\mu$  but near the apex 5-7.5 $\mu$  wide; bleeding when broken; when mounted in glycerine the contents break up into somewhat rectangular blocks (*Pl. VI, fig. 31*).

Tissue differentiation. - In structure the

fructifications are about 300-400 (600)  $\mu$  thick and the following regions can be differentiated:

(a) a hymenial layer up to about 50  $\mu$  thick;

(b) an intermediate hyphal layer measuring 150-250  $\mu$  thick, and composed of densely arranged hyaline hyphae, from this layer coloured conducting organs originate and curve upwards into the hymenium;

(c) a narrow golden yellow zone, about 50  $\mu$  wide lying between the intermediate layer and the outermost hairy covering, composed of longitudinally arranged hyphae; and (d) the outermost hairy covering composed of densely matted hyphae, about 50-100 (200)  $\mu$  thick.

Hyphal characteristics. - Two kinds of hyphae can be recognised: (1) thick-walled, matted or loosely interwoven, hyaline hyphae of the hairy covering, more or less straight, sometimes slightly flexuous, mostly unbranched, distantly septate, lumen often almost obliterated, without clamp-connections, about 2.5 - 5 (7.5)  $\mu$  wide and sometimes with a few granular contents, and (2) comparatively thin-walled interwoven or longitudinally arranged hyphae of the intermediate layer and golden yellow zone, sparingly branched, distantly septate, without clamp-connections, sometimes pale yellow in colour, with granular contents, about 3-5  $\mu$  wide.

The fructifications of this fungus develop readily on freshly felled poles of infected trees during the months of October to January. They are found either on the cross-section-ends over the rotted areas or on the cracked bark. They are commonly recognised in having thin, coriaceous and resupinate fruiting bodies and with a narrowly turned back margin. The hymenium, somewhat drab in colour, bleeds red when wounded and cracks irregularly and deeply on drying. Numerous coloured lactiferous cells in the hymenium and underlying tissues are conspicuous diagnostic characters by which the species can be readily identified. Owing to their small size they are difficult to recognise from a distance and as such, of little help as an indication of decay in standing trees in the forest. According to Burt (1920) and Rea (1922) there are no cystidia but recently Wakefield and Dennis (1950) have mentioned the presence of yellowish brown cystidia in the hymenium. They have however, not stated anything about the presence of numerous lactiferous cells so characteristic of the species and as such yellowish brown cystidia described by them are in reality the conducting organs of the previous workers. These organs bleed i.e., their contents come out when broken and this is the unique character of the conducting organs and not of cystidia as given by Overholts (1929).



2. PLEUROTUS MITIS (Pers.) Berk.(Plate III Figs. 13-15)

PILEUS. - Sub-sessile or shortly stipitate, continuous with the stipe in a more or less horizontal plane; thin, slightly fleshy, tough and flexible; sub-spathulate to somewhat reniform; about 5-15 (2)m.m. across.

UPPER SURFACE. - At first entirely white, later becoming slightly rufous or showing shades of salmon colour or flesh ochre near about the stipe; smooth, glabrous, often showing fine longitudinal striations near the margin; margin entire, involute when young or on drying.

FLESH. - Thin; sub-gelatinous below the cuticle; white; less than .5 m.m. thick.

GILLS. - Very narrow, linear-lanceolate; crowded; adnate; white, becoming pale salmon colour with age or on drying; about 1-1.5 m.m. wide.

STIPE. - Short, lateral, widening into the cap; white or tinged rufous; somewhat compressed; covered all over with whitish, mealy squamules; about 1-3 m.m. long, sometimes becoming much elongated (6-12-15 m.m.) when developed within cracks, about 1-1.5 m.m. thick.

BASIDIA. - More elongated than the immature ones;



narrow clavate; with 2-4 strigmata  
 (1.5-2  $\mu$  long); dimension about 16-20-25 x  
 3.5-4  $\mu$ ; immature basidia about 9.5-12.5x  
 3-3.5  $\mu$  (text-fig. I, i)

SPORES. - Sausage-shaped; hyaline; smooth;  
 dimension about 4-5x1.5-2-2.5  $\mu$  (text-fig. I, h)

TISSUE DIFFERENTIATION. - A vertical section through the upper fleshy portion of the pileus shows three distinct regions; (a) the outermost brownish cuticular layer, about 20-30  $\mu$  thick, (b) an intermediate sub-gelatinous layer, about 180-200  $\mu$  thick, with loose stainable hyphae embedded in a gelatinous matrix, and (c) a more or less brownish basal portion, about 80-100  $\mu$  thick. In a cross section of the stipe three different regions can be distinguished, viz; (a) a dark compact and circular central zone, about 900-1200  $\mu$  thick and consisting of densely interwoven hyphae, (b) an intermediate hyaline sub-gelatinous zone, about 200-300  $\mu$  thick and with loosely interwoven hyphae and (c) the outermost compact zone of about 150-200  $\mu$  in thickness. A transverse section of the gill shows a central sub-gelatinous trama, about 60-150  $\mu$  wide, composed of compact parallel hyphae and its central axis loosening from the sub-hymenium and hymenium.

HYPHAL CHARACTERISTICS. - Two kinds of hyphae appear to have entered into the composition of the fruit-body, viz., (i) thin-walled, hyaline, wider hyphae, straight and more or less parallel, sometimes slightly flexuous, distantly branched and septate, with a few granular contents, sometimes empty, about 2.5-4(5)  $\mu$  wide, without clamp-connections and with walls often partially gelatinised, and (ii) thin-walled, hyaline, narrow hyphae, about 1.5 to 2.5  $\mu$  wide, flexuous sparingly branched, with clamp-connections, full of granular contents and more or less inter-twined. These two kinds of hyphae are characteristic of the gills and the stipe. In the gill the wider hyphae are more numerous than the other type and appear to run more or less parallel within the trama. The narrower hyphae, on the other hand, are highly flexuous and embedded in a gelatinous matrix within the stipe. The fleshy portion of the pileus is entirely made up of this type of narrow fluxuous hyphae similarly embedded in a gelatinous matrix, the wider hyphae being unrecognisable as a result of gelatinisation.

This fungus, long called Agaricus mitis Pers., is well known in France, Switzerland as well as in all of Europe and is frequently found on dead twigs and stumps of coniferous trees. Berkeley (1860)

mentions that it occurs on larch in Scotland and is abundantly found in Nottinghamshire. I have collected it growing on dead twigs and stumps of Norway spruce, Sitka spruce and Douglas fir from Peebles, Scotland. It has also been collected while growing saprophytically on Scots pine in this country.

Josserand and Smith (1937) state that this species is identical with the American species Panus bacillispora Kauffman and suggest that these two names should fall into synonymy with Panellus mitis (Pers.) Singer. In order to avoid confusion, I have retained for the present the older name Pleurotus mitis by which it is familiarly known in this country and on the continent of Europe. Pleurotus mitis is easily recognised by its tiny, white fructifications which later become somewhat rufescent. Though not externally recognised, its gelatinous flesh and trama and narrowly allantoid spores are distinctive characters. The fructifications revive after a period of dessication and are gregarious.

#### (d) FUNGI in CULTURE.

The isolates of S. sanguinolentum and P. mitis from Norway spruce were grown in culture and their characteristics studied on two different media, viz., potato-dextrose agar and malt agar. Potato-dextrose agar was prepared according to the recipe of Fritz (1923). Culture tubes (6" x  $\frac{3}{4}$ ") were used for

Jaag (1937) determined the maximum, optimum and minimum temperatures for the development of the fungus on malt agar. The descriptions based on cultures of the isolates from spruce in this country have been given in the following pages.

#### 1. OXIDASE TESTS.

There are evidences in support that the intensity of attack on lignin is directly dependent upon the oxidase activity of the white rot fungi and this can be detected by the method described by Bavendamm (1928). This oxidase test was made by growing the fungi in Petri-dishes on 2.5% malt agar containing 0.5 per cent of gallic or tannic acid and kept at a constant temperature of 23°C in darkness. Dark brown rings appeared within 24 hours around the inocula of both the fungi indicating the presence of oxidase. This positive reaction indicated that both the isolates of S. sanguinolentum and P. mitis were white rot fungi but their intensity of reaction showed some variation. On gallic acid medium within 24 hours after inoculation P. mitis showed very intense reaction and the diameter of the ring was 20-25 m.m., whereas the diameter of the same produced by S. sanguinolentum was only 10 m.m. and the reaction was less intense than the other (Pl. IV, V, figs. 17, 22). On tannic acid medium the reactions were intense to very intense in both



cases. Another method to distinguish white rot fungi from brown rot ones (Preston and McLennan, 1948) was successfully tried by growing both the fungi on 2.5% malt agar containing 0.007 per cent gentian violet. Being white rot fungi, the violet colour of the medium was bleached slowly by them and about 6 to 8 weeks incubation was necessary before definite positive results could be obtained. According to Preston and McLennan complete decolorization of the dye would take place only when the oxygen concentration above the medium was greater than 6% and this discolouration was probably due to the production by the white rot fungi, of an extracellular oxidase system.

## 2. CULTURAL CHARACTERISTICS.

### A. Stereum sanguinolentum (A. et S.) Fr.

(1) Habit of growth (Pl. IV figs. 18-21) On potato-dextrose agar at 18°C the growth of the mycelium started with moderate rapidity and it was initially thin and arachnoid with an even advancing zone. During the first seven days after inoculation it remained in this condition excepting on the transplant where a shaggy ball was most often present. As the growth advanced, the mat proper at first glabrous, gradually became denser and covered the whole surface of the slant with soft, downy-woolly mycelium, somewhat thin, appressed and fringed at

the margin. In about 12 days after inoculation the mycelium tended to fill the base of the tube with a compact felty mat which in the older part, was depressed and somewhat opaque. The new growth was downy to cottony-woolly and somewhat translucent, particularly towards the upper end of the tube. The colour of the medium changed to yellowish with a pinkish tinge. Yellowish glistening drops of liquid appeared over the inoculum and on the mat in about 3 weeks' time but later, these disappeared. At 23°C the growth was more or less the same but the condensation of the mat seemed to be more pronounced. On malt agar under identical conditions the growth was rather slow, thin and arachnoid at the beginning but somewhat cottony to felty over the inoculum and not a shaggy ball. As the growth advanced the mat became felty, patchy and in about a month, formed a tough skin-like mat, difficult to tear. In general, growth and subsequent condensation of the mycelium to form a thick mat was less pronounced in malt agar than that in potato-dextrose agar.

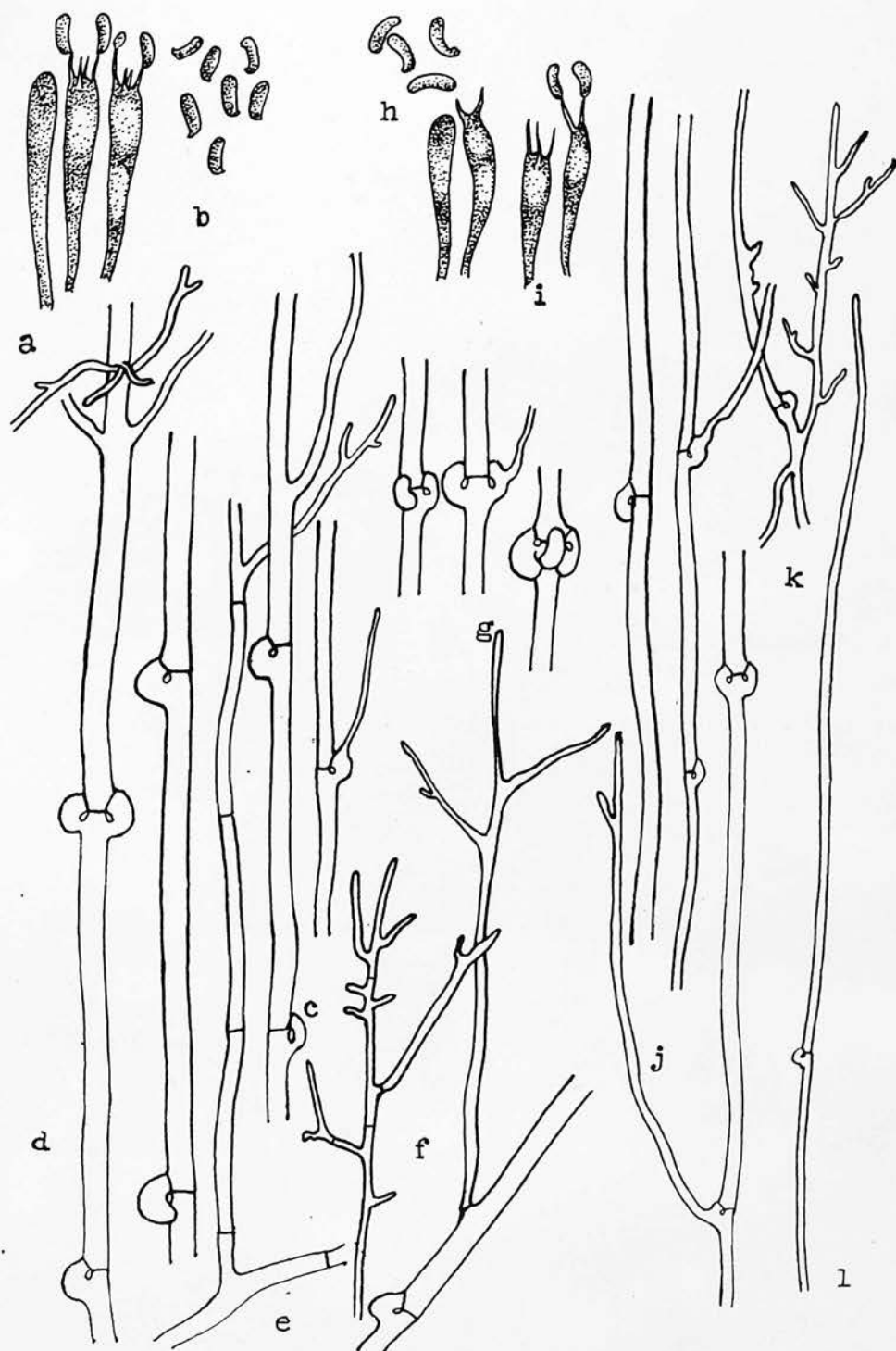
(ii) Colour - On potato-dextrose agar at 18°C colouration appeared first after a week's growth over the inoculum as a patch of Apricot buff which later deepened to Buckthorn brown or Mummy brown. Within a fortnight after inoculation, pigmentation of the mat at 23°C was confined to the area just around the

inoculum and included shades of Antimony yellow, Buckthorn brown and Mummy brown, while the major portion of the mat remained white. Within a week after this, diffused Antimony yellow colour appeared over the whole mat but it was more pronounced towards the lower end of the slant which later deepened to patches of Ochraceous orange. Although pigmentation started early at 23°C, later it was more pronounced in cultures kept at 18°C. On malt agar at 18°C colouration of the inoculum and the surrounding mat started about 12 days after inoculation and was Pinkish buff in colour. Later other shades such as Warm buff, Antimony yellow and Ochraceous orange developed on the mat. These were restricted to limited areas of depressed mycelium of a denser texture. At 18°C colouration of the mat seemed to be more pronounced than that at 23°C and also more pronounced than that at 18°C and 23°C on potato-dextrose agar.

(iii) Rate of growth - The increment in diameter of Petri -dish cultures on 2.5% malt agar at 23°C in darkness were determined as follows:-

Days	2	4	6	8	10	12
Average diameter of growth (m.m.)	15	30	45	60	75	90

These figures indicate that the fungus grows with moderate rapidity, with an average daily



Text-fig. I. Basidia, basidiospores and aerial and submerged hyphae of *S. sanguinolentum* (a - g) and *P. mitis* (h - l). (c-g, j-l,  $\times 650$ ).



increment in diameter of 7.5m.m. At 18°C on the same medium the growth was slightly slower with an average daily increment of growth of 7 m.m. On potato-dextrose agar, on the other hand, the rate of growth at 23°C was much more rapid than that on malt agar with an average daily increment in diameter of 8.5 m.m.

(iv) Hyphal characters (text, fig.  $I_n^{c-2}$ , Pl. VI figs. 26-29).

Advancing zone: hyphae hyaline, straight or flexuous, sparingly branched, usually distantly septate, with frequent large, simple clamp-connections, sometimes in opposite pairs or whorled, about 3-6(7)  $\mu$  wide, with conspicuous granular contents, sometimes pale yellow in colour.

Aerial mycelium: three types of hyphae can be recognised, viz., (1) wider thin-walled hyphae, more or less straight or slightly flexuous, usually distantly septate, with a few simple or paired, arched clamp-connections, very sparingly branched, usually 5.5 - 6.5  $\mu$  wide but may be up to 7.5 - 8  $\mu$  broad, sometimes with yellowish-brown contents, often giving rise to finer hyphae from the sides which may be clustered at the septa (Text. fig.  $I, d, g,$ ); (2) thin-walled narrower hyphae, sparingly branched, distantly septate, rarely with simple clamp-connections which may grow out to form new hyphae, about 2.5 - 4.5  $\mu$  wide, often spirally coiled (Pl. VI figs. 26, 27); with

or without granular contents, hyaline or brownish in colour (Text-fig. I.c.e); (3) very narrow, thin-walled, hyaline, much branched hyphae originating from the wider or intermediate types, often flexuous, with dense, granular contents, without clamp-connections (Text-fig. I.f ).

Submerged mycelium: The same three types of hyphae can be recognised but with slight differences as follows - (1) wider hyphae very numerous, hyaline, mostly with granular contents, about 5.5 - 6.5  $\mu$  wide, often slightly constricted at the septa, without clamp-connections; (2) intermediate type fairly numerous, sparingly branched, branches flexuous, with or without granular contents, hyaline or faintly coloured, distantly septate, rarely spirally coiled; (3) finer hyphae very few. Crystals large, numerous.

B. PLEUROTUS MITIS (Pers.) Berk.

(i) Habit of growth (Pl. V figs. 23-25). On potato-dextrose agar at 18°C (fig. 23 a) the growth was at first slow being only confined to the inoculum as a dense, white and felty covering. As the growth advanced, the mycelium condensed over it forming a felty ball while on the agar surface it remained entirely appressed, sodden and colourless with indistinctly fimbriated margin. In about 10 days

after inoculation, the mat proper became differentiated into a broad central, white and felty zone around the inoculum and a distinct appressed and colourless advancing zone, about 2-3 m.m. wide. In cultures about 20-days-old the mat presented the appearance of a dense, white and smooth felt while the region just behind the advancing zone tended to become somewhat raised and sub-felty. In old cultures both the central and advancing zones became felty but were separated from each other by a narrow sub-felty area and eventually a thin skin was formed. Considerable discolouration of the medium was noted in cultures 10-days-old and it was pale yellow. At 23°C (fig. 23c) on the other hand, the habit of growth was more or less the same but the rate of growth was rather slow and the mat was more compact. On malt agar, both at 18°C and 23°C (figs. 23c,d) the growth was poorer and the mat proper remained mostly appressed and colourless for a considerable length of time. Towards the end of the second week after inoculation a felty to pulverulent central zone was differentiated and an outer appressed and colourless zone of variable width (2-8 m.m.) could be clearly recognised.

(ii) Colour - On both the media all cultures remained white throughout and no other pigment developed even in cultures 2 to 3 months old.

(iii) Rate of growth - The rate of growth on both the media, under identical conditions of light and temperature as stated before, was rather slow to start with but after a week it increased and the daily increment in diameter of Petri-dish cultures were determined. The radial growth in all cases was unequal and as such average measurements were taken. On malt agar both at 18°C and 23°C (Pl. V figs. <sup>24,25</sup>) the daily increment of growth varied between 6.5 and 7 m.m. while on potato-dextrose agar, it varied between 5 and 7 m.m.

(iv) Hyphal characters (Text fig. <sup>j-l</sup> I, Pl. VI fig. 30)

Aerial mycelium; hyphae hyaline, more or less straight or slightly flexuous, remaining unbranched for a considerable distance (text-fig. <sup>j</sup> I, j ) but often becoming very flexuous and closely branched, (text-fig. <sup>k</sup> I, k ) usually with simple clamp-connections at every septum, paired clamp-connections few, often developing into new hyphae, mostly 1.5 to 3  $\mu$  sometimes 3.5 - 4.5  $\mu$  wide, with dense to sparsely granular contents, sometimes empty.

Sub-merged mycelium: hyphae hyaline, sparingly branched, usually flexuous, simple clamp-connections (Text fig. <sup>l</sup> I, l ) frequent but not so numerous as those in aerial hyphae, distantly septate, mostly 3 - 3.5  $\mu$  sometimes 1.5 - 2.5  $\mu$  wide; a few prominent flexuous usually unbranched hyphae with densely granular, highly refractine contents present, without clamp-



connections, about 5 - 7.5  $\mu$  occasionally 3.5  $\mu$  wide; on being mounted in lectophenol its contents become more or less homogeneous and the walls of the hyphae bulge out at places to form lateral swellings (Pl. *VI* fig. 30).

(e) PRODUCTION of FRUIT-BODIES.

It has already been stated that the two fungi isolated from the infected trees were identified as *S. sanguinolentum* and *P. mitis* respectively, by studying and comparing their cultures with the standard cultures of the same already made available for the purpose. Since the identification of unknown cultures largely depends upon their forming characteristic fructifications, various attempts were made to obtain these in pure cultures of the two isolates so that they could be easily recognised. Both the isolates however, did not form fructifications on various media tried such as potato-dextrose agar, malt agar, oatmeal agar and bread which were subjected to various conditions of moisture, light and temperature within the laboratory. Only *S. sanguinolentum* formed small resupinate fructifications on sterilized wood blocks of Norway spruce within 2 to 3 months after inoculation (Pl. *VII* fig. 34). These wood block cultures were kept in darkness and at a constant temperature of 23°C in an incubator. Similar wood block cultures of *P. mitis* did however,

remain sterile. Eventually the medium for developing sporophores recommended by Badcock (1941) was tried with considerable success. Dry sawdust of readily decaying wood such as beech and spruce were separately mixed with Badcock's 'accelerator' in various proportions (5% to 40% by weight), the principal ingredients being maize-meal and bone-meal. The mixtures after being soaked in tap water, were put in Petri dishes, test-tubes (20x4 c.m. and 15x2 c.m.) and Badcock's apparatus (1943) and the various devices described by him for obtaining an atmosphere of relatively high humidity around the developing sporophore, were tried. This was found to be necessary as Badcock rightly pointed out that no single method could be expected to induce fructifications in all species of wood-rotting fungi equally, for they behave very differently under natural conditions. After sterilization the media were inoculated and the cultures were kept in darkness at room temperature ( $18^{\circ}\text{C}$  to  $21^{\circ}\text{C}$ ) inside a closed chamber in which a moderately high humidity was maintained. Subsequently, when the mycelia entirely covered the surface of the medium or reached the ends of the tubes, all the cultures were removed and placed in strong diffused light on a table about 8 ft. from a large window of the laboratory. Both the fungi produced exceptionally rapid and luxuriant growth on the sawdust medium,

particularly on those containing 30% or 40% by weight of the 'accelerator'.

In all cases, S. sanguinolentum began to form either resupinate or typical effuso-reflexed fructifications within 2 to 3 months after inoculation (Pl. VII fig. 33). When the medium in large tubes was completely permeated with compact mycelium, the cotton plugs were removed and each was supported in an inclined position between two Petri-dish lids, the lower being filled with water. A thick pad of absorbent cotton wool was placed within the mouth of the tube and was never allowed to dry out. Within a few days, when vigorous surface growth developed, the pad was gently pushed down so as to touch the medium. The tube was then placed in such a position so that light from the window could fall directly on its open mouth. In this way several effuse-reflexed fructifications were obtained in about 5 to 6 months after inoculation. Although there was risk of contamination, this method proved to be very successful. In Badcock's apparatus small resupinate fruiting bodies developed near the rim of the test-tube within the flask but the growth was very slow. Cultures in Petri dishes in an advanced stage of growth were exposed by removing the lids to humid atmosphere and these also fructified and produced resupinate fruiting bodies within 2 to 3 months

after inoculation, particularly near the edge of the lid. It was noted that in case of S. sanguinolentum the medium containing 5% 'accelerator' was found to be most satisfactory for the production of sporophores. The higher the percentage of the 'accelerator' the greater was the vegetative growth with consequent delay in producing fruiting bodies.

Great difficulty was, however, experienced in obtaining fruit-bodies of P. mitis although various devices recommended by Badcock were tried. Under laboratory conditions of light and temperature the mycelium covered the surface of the medium with white and compact growth and crept over the rim of the lower lid of the Petri dish forming somewhat compact, tough, irregular and skinlike mat. At this stage the upper lids were carefully removed and the cultures were kept inside a bell-jar in which a relatively high humidity was maintained by keeping a dish of water exposed within it. Within a fortnight short stalks began to develop near the edge but these did not expand further to form pilei. About the middle of October 1950 some of these cultures (about 4-5 months old) with rudimentary stalks were kept in the open outside the laboratory, under a moist bell-jar below a shade, in order to avoid direct sunlight. A wad of moist absorbent cotton wool was placed on the culture in close contact with the undeveloped stalks and never allowed



to dry out. The temperature was daily recorded and it varied between 5°C and 15°C till the middle of November. Within a fortnight numerous stalks began to develop on the outer edge of the skinlike mat and within a month their tips expanded and formed pilei with rudimentary gills on their under surface. (Pl. VII figs. <sup>35,36</sup>). In this way several crops of fructifications of P. mitis were obtained. It was found that temperatures of the laboratory (18°C - 21°C) and incubators had a retarding influence on the production of sporophores. Given all other necessary conditions, a range of temperature varying between 8°C and 15°C was found suitable for their development. When the temperature fell below 8°C development of fructifications was gradually stopped. P. mitis appeared to be very sensitive to temperature conditions and it was also necessary to increase the amount of 'accelerator' to 40% by weight for ready development of the sporophores. Fructifications of both the fungi when sectioned showed well-developed hymenia with normal basidia and basidiospores.

Fructification of both S. sanguinolentum and P. mitis began to develop readily when small sections of freshly felled infected trees were brought into the laboratory and kept outside in the open, during the months of November to January.

(Pl. I, III figs. 4, 11, 12). Occasional showers kept the poles moist and a comparatively low temperature was found to be suitable for their development. In this respect P. mitis appeared to be very sensitive to outside temperature for it was also observed that a range of temperature between 8°C and 15°C was found favourable for their development. During the rest of the months the fungi lived well in the moist wood but did not fructify at all. Similar infected poles were also kept moist in diffused light and temperature (18°C - 21°C) of the laboratory. P. mitis formed dense strands of white mycelium on the surface of the wood (Pl. VII fig. 37), but did not produce any fruit-body. Small resupinate fructifications of S. sanguinolentum however developed at the cross-section-ends of the poles but their growth appeared to be very slow.

#### IV. DECAY OF SPRUCE.

##### A. Microscopic Studies.

In order to find out the extent and effects of the mycelium within the host, pieces of wood in various stages of decay were sectioned both free hand and with the microtome. In most cases transverse, radial and tangential sections of the wood, about 15-20  $\mu$  thick, were cut without any

special treatment, from freshly felled poles in order to avoid drying out of the wood and consequent shrivelling up of the fungal hyphae. Sections from partially dried wood were obtained only after some kind of softening treatment and this was done by immersing the blocks in a vessel of gently boiling water until they became water logged and sank. After the softening process the blocks were taken out and sectioned immediately while their surfaces had been kept wet with water or alcohol during the process. It was found that such sections were not very satisfactory since the mycelium within the dried wood was usually shrivelled or changed in some way or other so as to become somewhat invisible and thereby making observations more difficult. The sections were preserved in 50% alcohol for future use. For ordinary purposes free hand sections were found to answer the requirements well to ascertain the presence of mycelium within the host tissue. Since it was very difficult to study hyphae in detail within sections of wood, differential stains such as those recommended by Hubert (1922) Cartwright (1929) and others were tried but the method proposed by Cartwright was found to be very satisfactory in rendering the hyphae visible and easy to manipulate.

In an early stage of decay caused by S.

sanguinolentum, the mycelium was often scanty but later it was distributed throughout all the elements (Pl. VIII fig. 41) being particularly plentiful in the medullary rays which seemed to be the first element of the wood to be attacked. The hyphae, sometimes filling entire cavities of the elements (Pl. VIII fig. 41) were of two kinds; firstly, rather fine, profusely branched, hyaline, about  $1-2\ \mu$  across and secondly, a coarser hyphae (Pl. IX figs. 42-44) of about  $3-5\ (6)\ \mu$  wide. The larger hyphae were hyaline or pale yellow in colour, occasionally with reddish-brown contents and usually bearing large, simple or double clamp-connections (Pl. IX figs. 43, 44). Similar double clamp-connections on coarser hyphae were recorded in two samples of spruce by Jorstad and Juul (1931) but have not so far been observed in the wood by Cartwright and Findlay (1948). Clamp-connections were rare on the finer hyphae. The finer hyphae appeared to be more numerous and probably were the first to invade the uninfected parts. These could be definitely demonstrated in properly stained preparations even in an incipient stage of decay in which the coarser hyphae with double clamp-connections had not been observed. The hyphae at first ramified through the pits but later directly through the cell-walls forming numerous bore-holes (Pl. X fig. 50). The much branched finer hyphae in passing through the



cell-walls exhibited little or no diminution in diameter while the larger hyphae when passing through the cell-walls, became markedly attenuated or constricted towards the apex. The photograph (Pl. X fig. 45) submitted in evidence shows clearly the bore-holes slightly wider in diameter than the contained hyphae; these bore-holes were formed in advance by enzyme action at the point of contact of the hyphae. The bore-holes were usually circular or somewhat cylindrical and plentiful in an advanced stage of decay. Many of the ray parenchyma cells and resin canals (Pl. V fig. 32 ) were filled with closely interwoven mycelium associated with a yellowish gum-like substance. These were also occasionally present in the tracheids. Similar plugs of gum-like materials giving the appearance of a zone-line had also been observed by Cartwright and Findlay (1946). In passing through the bordered pits the hyphae destroyed the closing membranes, thus enlarging the opening. In an advanced stage of decay the walls of the bordered pits were dissolved by enzyme action as shown by the smooth contours of their surfaces and their circular form. The wood in an advanced stage of decay (Pl. X fig. 48 ) showed that the secondary walls of the tracheids had been gradually dissolved outwards towards the middle lamella and this appeared to be a slow process.

The microscopic details of the rot caused by

P. mitis were more or less similar to those caused by S. sanguinolentum except for the following differences. The hyphae were exceedingly narrow, much branched, hyaline, about 1-1.5 m.m. across and plentiful in the decayed wood (Pl. VIII figs. 39, 40) but slightly wider hyphae up to 3  $\mu$  wide were also present. Simple clamp-connections (Pl. VIII fig. 40) were very numerous, almost at every septum; the modes of hyphal penetration were through the pits as well as through the cell-walls (Pl. VIII fig. 40). The bore-holes (Pl. VIII fig. ) exceedingly small, more or less circular in outline, were very numerous. The walls of the bordered pits (Pl. VIII fig. 38) were broken down early. Entangled masses of hyphae were quite common in the tracheids. The ray parenchyma cells were filled with interwoven hyphae and yellowish or brownish gummy materials but the latter were rare in the tracheids.

#### B. Microchemical Studies.

Microchemical tests for lignin and cellulose in the sound and partly decayed wood (Pl. X figs. 47-49) have been recorded. This was done in the usual way by staining the sections of wood with phloroglucin and hydrochloric acid for lignin

and with chlor-zinc-iodine for cellulose. The combination stain, safranin and fast green which differentiates lignified (red staining) and unlignified (green staining) structures and has superseded the old safranin and haematoxylin combination, was tried. Various other microchemical stains were also employed, most of which agreed closely with the changes indicated by the use of above-mentioned stains. It is not wished to draw any definite conclusion from these stain-reactions alone, since it has been recently shown (Harlow 1928; Hirt 1927) that these microchemical stains are not infallible indicators of the chemical changes that take place during the process of decay in the wood. Nevertheless, these stains are still used in such work to indicate the presence of lignin and cellulose and the results thus obtained are tabulated in the following pages.

Table I/

Table 1. Results of staining with safranin and fast green for cellulose and lignin in normal and partially decayed wood of Norway spruce.

Elements	Cellulose		
	Normal Wood	Wood decayed by <u>S. sanguinolentum</u>	Wood decayed by <u>P. mitis.</u>
Wood tracheids.	Secondary walls surrounding the lumen either green or partly green and extending up to middle-lamella, occasionally almost entirely green; bordered pits often faintly green surrounding the aperture.	Secondary walls round the lumen faintly green, often partly light green, and extending up to middle-lamella.	Secondary walls <sup>in the</sup> summer-wood occasionally, partly pale green; in spring wood mostly pale green round the lumen; bordered pits often greenish.
Ray cells.	Secondary walls often partly greenish	Occasionally faintly green or colourless.	Occasionally colourless.
Resin ducts.	No reaction	No reaction	No reaction

Lignin/



Table 1. continued....

Elements	Lignin		
	Normal Wood	wood decayed by <u>S.sanguinolentum</u>	Wood decayed by <u>P.mitis</u> .
Wood tracheids.	Primary walls deep red; secondary walls light- er red; bordered pits mostly pink.	Primary walls red; secondary walls mostly pink towards the middle- lamella.	Primary walls red; secondary walls almost wholly light red to pink, occasionally partly so; bordered pits often pinkish.
Ray cells.	Primary walls red; secondary walls light red.	Primary and secondary walls mostly pink.	Primary walls red or light red; secondary walls mostly light red to pink.
Resin ducts.	Red	Pink	Light red.

In the normal wood the middle lamellae of all the elements stained red. The secondary walls of the wood tracheids were partly light red and partly green but sometimes entirely green. The secondary walls of the ray cells were however, occasionally partly green. The secondary walls of the epithelial cells of the resin canals were so thin that possibly the red tinge came from heavily stained middle-lamellae. The bordered pits were mostly pinkish but with a faintly greenish tinge near the aperture.

In the decayed wood a light red to pink colour was more noticeable over the entire sections. The middle-lamellae remained red but they were light red to somewhat pinkish in the ray and epithelial cells of the resin canals. The secondary walls of all the elements were predominantly pinkish but occasionally light red, and faintly greenish to almost colourless surrounding the lumen. The bordered pits were often entirely greenish in colour.

Certain investigators in using safranin and fast green as a combination stain state that the red colour indicates the presence of lignin and that the unligified tissues are stained green.

Accordingly, it can be broadly interpreted that the fungi in question gradually delignify all the elements but also utilize to a great extent the cellulosic materials of the wood. It would be hardly wise to draw any definite conclusion from the above staining reactions, since when other stain combinations such as gentian violet and Bismarck brown are used the partly brownish colour of the epithelial cells do not necessarily indicate lignin.

Table 2/

Table 2. Results of staining for cellulose and lignin in normal and partly decayed wood of Norway Spruce with chlor-zinc-iodine and phloroglucin-HCl respectively.

Elements	Cellulose		
	Normal wood	wood decayed by <u>S.sanguinolentum</u>	wood decayed by <u>P.mitis</u> .
Wood tracheids.	Secondary walls near the lumen mostly blue, sometimes light blue, in summer-wood less distinct; primary walls occasionally faint blue; bordered pits partly faint blue in most cases.	Secondary walls near the lumen light blue mostly, particularly in the spring wood; primary walls occasionally partly light blue; this reaction more conspicuous than that in normal wood; bordered pits faint blue at places.	Secondary walls partly faint blue at places; reaction more feeble than that in normal wood; primary walls at places faint blue in spring wood
Ray cells.	Secondary walls partly faint blue particularly of the ray tracheids.	Secondary walls partly light blue.	Secondary walls mostly colourless.
Resin Ducts.	Partly faint blue.	No reaction	No reaction.

Lignin/



Table 2 continued.....

Lignin			
Elements.	Normal wood	Wood decayed by <u>S. sanguinolentum</u>	Wood decayed by <u>P. mitis</u>
Wood tracheids	Primary walls deep red; secondary walls lighter red near the middle- lamella to pink near the lumen; often entirely light red or pink; bordered pits pink.	Primary walls red or pink; secondary walls mostly pink; light pink particularly near the rays; bordered pits pink or colourless.	Primary walls red or pink; secondary walls light red or pink, occasionally faint pink; bordered pits often colourless near the aperture but mostly pink.
Ray Cells	Primary walls red; secondary walls pink.	Primary walls pink; secondary walls pink; occasionally both colour- less.	Primary walls pink; secondary walls light pink; occasionally both faintly pink.
Resin Ducts.	Mostly red.	Pink, occas- ionally red.	Mostly red.

Phloroglucin - HCl. - In normal wood the middle lamellae of all the elements stained a deep red. The secondary walls changed from light red near the lamellae to somewhat pink near the lumen. Occasionally, they were entirely light red or pink. The secondary walls of the ray cells and the walls of the bordered pits were pink. The epithelial cells of the resin-canals remained red.

In the decayed wood the middle-lamellae of the wood tracheids remained red or became pink and those of the ray cells mostly pink. The secondary walls of the wood tracheids and ray cells mostly became entirely pink or lighter in colour but occasionally remained light red. The middle-lamellae and secondary walls of some of the ray cells became colourless. The walls of some of the epithelial cells turned pink.

Chlor-zinc-iodine - In normal wood the middle-lamellae seldom showed faint blue colouration. The secondary walls of the wood tracheids were mostly blue or light blue and those of the ray cells, faint blue. This was particularly the case with the ray tracheids. In the summer wood this reaction was less distinct. The walls of the epithelial cells were partly faint blue.

In the decayed wood occasionally the middle-lamellae remained partly faint blue. In wood

decayed by S. sanguinolentum the secondary walls of the wood tracheids showed intense cellulose reaction. They were mostly light blue, particularly in the spring wood and partly light blue in ray cells. The bordered pits were faint blue, particularly in the spring wood. In wood decayed by P. mitis, on the other hand, the secondary walls of the wood tracheids showed feeble cellulose reaction, being partly faint blue at places. The secondary walls of the ray cells were mostly colourless. The bordered pits remained colourless in both cases.

The pink colouration of the secondary walls of the wood tracheids and ray cells when treated with phloroglucin-HCl and the blue or light blue colouration in response to chlor-zinc-iodine, indicate that they are only moderately lignified. The chemical changes indicated by these staining reactions clearly show that there was delignification of the lignified parts of the secondary walls associated with the digestion of cellulosic materials of the wood. In wood decayed by S. sanguinolentum some of the secondary walls showed only pale pink colouration but mostly stained light blue throughout, suggesting partial removal of lignin from those walls. This reaction was more conspicuous than that in the normal wood. In case of p. mitis there was least

cellulose reaction and the walls turned red or pink, suggesting the presence of more lignin in those walls.

Sulphuric acid tests. - Sections of normal and decayed wood corresponding to those used for microchemical stains were placed upon slides and treated with 72% sulphuric acid as suggested by Ritter (1925). Immediately, in sections of normal wood, the cells swelled up rapidly and broke apart into fragments in the spring wood. The secondary walls of the wood tracheids became translucent and partly dissolved while the network of the middle lamellae remained intact in the summerwood but torn apart in the spring wood. The ray elements remained completely unaffected but their secondary walls became swollen and partly dissolved. The violent action during the process was due to the presence of considerable amount of cellulose in the cell-walls of the sound wood. The swelling of cellulose preceeding dissolution forced all the elements apart in the springwood.

In sections of wood decayed by both S. sanguinolentum and P. mitis fragments of secondary walls of the wood tracheids were dissolved leaving the network of middle lamellae and wood elements in place on the slide. In both cases the secondary walls of the tracheids became swollen, partly dissolved and semi-transparent. The



network of middle lamellae mostly remained in tact though broken off at places in the spring wood. There was much less distortion of the elements in springwood than those in the normal wood. The ray elements mostly remained unaffected and portions of their secondary walls were the only parts dissolved.

It may be concluded that the less violent action and consequently less distortion of the wood elements in the decayed wood were due to the presence of less cellulose than that in the sound wood. This is particularly true in case of wood decayed by P. mitis. Though not conclusive in itself, this test at least indicates the presence of less cellulose in the wood decayed by S. sanguinolentum and P. mitis respectively than that in the normal wood.

#### V. DECAY RESISTANCE TESTS IN THE LABORATORY.

In order to determine the natural resistance and progress of decay of spruce samples caused by S. sanguinolentum and P. mitis separately, under controlled condition in the laboratory, wood-block cultures of these fungi were made in the following way. In general, the method recommended by Baxter (1925) was employed with slight modification.

Small bits of actively growing mycelia were

transferred to sterile 2 per cent malt agar slants in 1000 cc Erlenmeyer flasks. Each flask contained 250 c.c. of the medium which was slanted after sterilization. After inoculation the flasks were incubated at 23°C in darkness. Within a week or so the mycelia covered the surface of the slants and were ready to receive the wood blocks.

The wood blocks measuring 2" x  $\frac{1}{2}$ " x  $\frac{1}{2}$ " were obtained from sound wood of Norway spruce. These were planed and serially numbered on all faces with a soft lead pencil and dried to a constant weight in an oven maintaining a temperature of 60°C. A comparatively low temperature was preferred to avoid the harmful effects of rather high temperatures on the composition of the wood blocks. Before sterilization these test-pieces were thoroughly soaked in distilled water, three such pieces were put in each Roux tube having distilled water at the bottom, plugged and sterilized in an autoclave at 15 lbs. pressure for 10 minutes. The idea of sterilizing the wood blocks in this way was to keep the atmosphere around them more or less saturated during the process so that little loss of water could take place from the wood blocks and consequently lesser time was necessary for sterilization as pointed out by Chidester (1937,1939). The test-pieces were then taken out individually from the

Roux tubes by a sterile forceps and exposed to fungal action by placing them on cultures of S.sanguinolentum and P. mitis. These flasks, each containing six samples of wood were kept at a constant temperature of 23°C in darkness. (Pl. XI figs. 51,52).

In order to obtain a significant loss in weight the periods of exposure to fungal attack were 4 months and 8 months as recommended by Cartwright and Findlay (1946). After completion of the tests the flasks were opened, the superficial mycelium was carefully removed without damaging the wood-blocks (Pl. XI figs. 53,54), weighed and again dried to a constant weight in an oven at 60°C. The final dry weight subtracted from the original dry weight gave the loss in weight of the wood blocks due to decay in 4 and 8 months.

The average moisture content of the wood blocks before they were exposed to fungal action was determined by weighing another set of 12 blocks, soaked and sterilized in the same way. The final dry weights when subtracted from these gave the initial moisture content of the wood-blocks before the experiment. This was necessary to keep the moisture content of the wood-blocks much above the 'fibre-saturation-point' which was necessary for the activity of the wood-rotting fungi. In this way the moisture contents of the inoculated wood-blocks after the tests was also determined. The results obtained are given in Tables 3 to 7.



Table 3. Decay resistance of Norway spruce wood  
after 4 months' test exposed to S. sanguinolentum.

No. of blocks.	Initial oven-dry weight (Grms.) before test.	Final oven- dry weight (Grams) after test.	Loss	Loss %
1	3.655	3.190	.465	12.7
2	3.490	2.985	.505	14.5
3	3.136	2.606	.530	16.9
4	3.151	2.630	.521	16.5
5	3.409	2.865	.544	16.0
6	3.409	2.914	.495	14.5
7	3.530	3.046	.484	13.7
8	3.569	3.043	.526	14.7
9	3.752	3.217	.535	14.3
10	3.692	3.181	.511	13.8
11	3.494	3.020	.474	13.6
12	3.608	3.100	.508	14.1

Average loss in dry weight (%) ..... 14.6

(Range - 12.7 - 16.9 )



Table 4. Decay resistance of Norway spruce wood  
after 8 months' test exposed to S.sanguinolentum.

No. of blocks.	Initial oven- dry weight (Grms.) before test.	Final oven- dry weight (Grms.) after test.	Loss	Loss%
13	3.489	2.309	1.180	33.8
14	3.636	2.602	1.034	28.4
15	3.753	2.795	.958	25.5
16	3.648	2.653	.995	27.3
17	3.900	2.901	.999	25.6
18	3.368	2.244	1.124	33.4
19	3.412	2.258	1.154	33.8
20	3.620	2.748	.972	26.9
21	3.743	2.639	1.104	29.5
22	3.808	2.844	.964	25.3
23	3.530	2.540	.990	28.0
24	3.164	2.195	.969	30.6

Average loss in dry weight (%)..... 29.0

(Range - 25.3 to 33.8)

Table 5. Decay resistance of Norway Spruce wood after 4 months' test exposed to P. mitis.

No. of blocks.	Initial oven-dry weight (Grams) before test.	Final oven-dry weight (Grms) after test.	Loss	Loss%
1	3.378	2.940	.438	13.0
2	3.414	2.978	.436	12.8
3	3.490	3.057	.433	12.4
4	3.273	2.798	.475	14.5
5	3.635	3.269	.366	10.1
6	3.210	2.726	.484	15.1
7	3.632	3.214	.418	11.5
8	3.402	3.007	.395	11.6
9	3.578	3.112	.466	13.0
10	3.135	2.760	.375	12.0
11	3.472	3.049	.423	12.2
12	3.902	3.448	.454	11.6

Average loss in dry weight (%) ..... 12.5

(Range - 10.1 to 15.1 )

Table 6. Decay resistance of Norway Spruce wood after 8 months' test exposed to P. mitis.

No. of blocks.	Initial oven-dry weight (Grams) before test.	Final oven-dry weight (Grams) after test.	Loss	Loss%
13	3.473	2.719	.754	21.7
14	3.179	2.335	.844	26.5
15	3.464	2.538	.926	26.7
16	3.520	2.614	.906	25.7
17	3.794	2.944	.850	22.4
18	3.579	2.784	.795	22.2
19	3.408	2.554	.854	25.1
20	3.362	2.578	.784	23.3
21	3.759	2.843	.916	24.4
22	3.652	2.818	.834	22.8
23	3.813	3.048	.765	20.1
24	3.625	2.715	.910	25.1

Average loss in dry weight (%)..... 23.8

(Range - 20.1 to 26.7)

Table 7. Comparison of loss in dry weight of wood blocks of Norway spruce exposed to S. sanguinolentum and P. mitis respectively.

	After 4 months		After 8 months.	
	Mean	Range	Mean	Range.
<u>S.sanguinolentum.</u>	14.6%	12.7 - 16.9%	29.0%	25.8 - 33.8%
<u>P. mitis.</u>	12.5%	10.1 - 15.1%	23.8%	20.1 - 26.7%

When/



When the respective values of the loss in weight due to S. sanguinolentum and P. mitis are studied in a comparative way the distinction is obvious as shown in Table 7. It is evident that the loss in weight due to P. mitis is less than that caused by S. sanguinolentum. But the interesting fact is that the loss in weight after 8 months is double of that after 4 months in both cases. The loss in both cases is quite significant as the test pieces have lost a high percentage of their original weight during the test and it can be safely concluded that the wood has low resistance to decay. Accordingly, spruce wood falls under Findlay's (1938) 'non-resistant' group as this class undergoes average losses from 10-30 per cent during 4 months' test.

The initial moisture content of the wood blocks before the experiment averaged 158%. After 4 and 8 months' test it was found to be above 50% in both cases. The results are presented in the following table.

Table 8/

Table 8. Moisture content (average figure) of the wood blocks after the test.

	After 4 months.	After 8 months.
<u>S. sanguinolentum.</u>	57.8%	51.9%
<u>P. mitis.</u>	61.8%	54.3%

VI. INOCULATION EXPERIMENTS.

Inoculation experiments were carried out with young twigs in the laboratory and also with trees in the field. In the former case the method described by Brooks and Moore (1923) was followed. In this experiment, 2 to 3 yearsold twigs of Norway spruce, about 4" long, were kept with their ends in water in the laboratory, their upper ends were cut off and spore-suspensions of S. sanguinolentum and P. mitis in sterile water were applied separately to the freshly exposed surface and never allowed to dry out. These were placed under a bell-jar in which a relatively high humidity was maintained by lining its interior with moist blotting paper. Longitudinal sections through the inoculated ends of the twigs were made at intervals of 24, 48, 72 and 96 hours after the spores had been added. Sections cut after a period of 24 to 48 hours showed that the majority of the spores had germinated on the surface and had sent hyphae down into the elements of the wood. A few spores were however, found to enter through the cut ends of the tracheids and had germinated there. Sections cut at intervals of 72 and 96 hours indicated that the spores had formed vigorous mycelium and invaded all the elements of the twig. Since these twigs could not be kept in

an actively growing condition, their invasion by S. sanguinolentum and P. mitis could not be regarded as an evidence for their parasitic action on trees. Nevertheless, it can be said that if by chance the spores somehow alight on the surface of the wound, they can germinate under suitable conditions producing germ-tubes which in some way pass down into the elements of the wood.

Field inoculations on living trees were started in early March, 1950, at Glentress forest. Two inoculations were made on 50 to 60 years-old vigorously growing Norway spruce with each fungus and with equal number of controls below. The operation involved was to make a cross-incision through the bark on the trunk and the trees were inoculated by transferring actively growing mycelium from tube cultures into the wound by carefully lifting a flap of the bark still attached to it.

Prior to inoculation the surface of the bark was wiped out with absolute alcohol and subsequently washed with sterile distilled water in order to free the surface from contamination as far as practicable. The flap was then covered with moist, absorbent, sterile cotton wool with a piece of thick paper over it in order to prevent drying out and firmly bound with a string. Towards the end of March the trees were examined. After removing the string and



the cotton wool it was found that in both cases the trees seemed to have taken the infection. The inoculated areas were distinctly bulged out with conspicuous secretions of resin. The controls, on the other hand, remained flat, rather depressed and there was little resin flow. These trees were again inspected in July and it was observed that in both cases the controls remained flat and were healing up satisfactorily. There was very slight resin secretion within the cut. In case of S. sanguinolentum the wounds were gaping open, looked more or less swollen, somewhat spindle-shaped and there was copious secretion of resin running down the stem (Pl. XV figs. 35). From the appearance of the bark it looked as if the infection had spread on the left side about  $1\frac{1}{2}$ " from the point of infection. Infections with P. mitis also looked somewhat oval and the resin was running down the stem. The infection appeared to have spread about an inch from the point of infection. In November, after 8 months, the trees were examined and the progress of infection was noted. All controls healed up. In case of S. sanguinolentum the bark was gradually sinking and there was more resin flow from the infected wounds than those caused by P. mitis. From the outward indications it could be said that both the fungi had infected the living trees through wounds, although S. sanguinolentum seemed to be more active of the two pathogens and

produced more resin which ran down the stem. At this time no fruiting-bodies of the fungi were observed and this was only to be expected since both the fungi were slow growers within the tissues of the host.

In March, 1951, the trees were examined and one of them felled, <sup>(Pl. XII, figs. 56-57).</sup> The internal conditions of the inoculated areas were microscopically examined; mycelia of both Stereum sanguinolentum and Pleurotus mitis respectively were present in the elements of the wood. Copious gum formation had taken place within the medullary rays.

Macroscopic examination of the infection by Stereum sanguinolentum showed characteristic reddish-brown rot in the sapwood. The mycelium had spread approximately eight inches both above and below the point of infection (Pl. XII, fig. 58). Spread in the transverse direction was about half an inch. In the infection with Pleurotus mitis the yellowish-brown rot is confined to the sapwood and extended about two inches both above and below the point of inoculation; the spread in the radial direction is about one quarter of an inch (Pl. XIII, fig. 59).

VII. DISCUSSION.

This study has been undertaken to obtain information of a serious disease causing bark necrosis on the trunk on Norway spruce at Glentress forest, near Peebles, in Scotland. The injuries are restricted to the stem mostly within the pruning limits and have been diagnosed as having arisen from the attack of Stereum sanguinolentum and Pleurotus mitis; these fungi have entered through the dead branches or other injuries such as pruning cuts that might have taken place at the beginning or the end of the growing season. Although similar injuries on Norway spruce have been attributed by Day (1950) as entirely due to frost damage, there is no such evidence on trees under consideration. Gähmann (1950) has described similar injuries in Switzerland on the bark of Norway spruce and silver fir, in a situation exposed to frequent hailstorms and strong electrical discharges which killed strips of cambium infection with P. mitis followed. Careful examination of the infected trees and the anatomy of the injuries did neither reveal the presence of frost cracks and frost rings in their wood nor was there any sign of bark injury due to electrical discharges. In addition, during the past few years there is no evidence that frost injury has really occurred in the crop as a whole.

On the other hand, close examination of the wounds reveals that the cambium has been killed near the pruned branches and the indications are that the causal agents after entering through the wound in the dead branches or pruning cuts, have killed the cambium and spread from the periphery to the centre of the wood, thereby rendering the wood unfit for commercial purposes. In the absence of any other evidence, it may be taken that these wounds have served as initial portals of entry. Both the fungi are common in the plantation as saprophytes on fallen branches lying on the floor of the forest during the colder months of autumn and the early part of winter.

Low air-temperatures appear to be favourable for the development of the fruiting bodies and during the spells of mild weather, i.e., late autumn and early spring when the trees are in a dormant condition, the spores after being carried away by the wind alight on the cut surfaces of the pruned branches where they germinate and penetrate the wood. It has been experimentally proved that a comparatively low temperature is necessary for the development of the fruit-bodies, particularly in case of P. mitis, and that the spores can germinate within a short time on the cut surfaces and infect the twigs under moist conditions. Inoculation experiments on living



trees carried out in the field during early spring have shown that both the pathogens can infect standing trees through wounds in the bark and from the results of inoculation they can be regarded as wound parasites on normal vigorous trees. Although no infection experiments have been carried out with dead branches, it can be assumed that presumably they can also infect them as they can live well in the wood of the fallen branches. As long as the trees are in sap and the air-temperatures are high, the fructifications cannot develop and consequently there is comparatively less chance of any infection taking place during the period. In summer, the fungi thrive well in the dead branches in a moist cool situation. Experiments with infected trunks point to the conclusion that the fungi can live within the wood throughout the year and begin to fructify only during the months of October and November, when the outside temperature is low and favourable for their development.

Both S. sanguinolentum and P. mitis have been isolated from standing trees and found to be the cause of the disease. Previous investigators of spruce have failed to record that P. mitis can cause an important decay in wood of living trees in this country. In transverse sections of the infected poles, the general appearance of the rots

in the early stages of decay caused by S.sanguinolentum and P. mitis, is very similar and in this study both decays were at first thought to be caused by the former fungus until cultural and microscopic investigations proved differently. This has emphasized the importance of conducting cultural studies in support of decay determinations which are often based on gross appearance of the rots. It has, however, not been possible to find out whether both the pathogens have infected the trees at about the same time or whether one of them has entered first and weakened the tree, thus preparing the ground for a secondary infection with the other fungus. At the present moment it is difficult to estimate the relative importance of the two fungi in bringing about infection and decay in living trees and the nature of interactions existing between them but this association of two pathogens has no doubt greatly enhanced the importance and complex nature of the problem. However, both the fungi have been isolated not only from the same pole but the respective rots were only a few centimetres apart from each other, being separated by healthy tissues. From the number of isolations so far made, it is apparent that S. sanguinolentum is more widely distributed within the host tissues than P. mitis. Further investigations in future with large number of infected trees would possibly throw some light as

to their distribution within the host plant. In consequence, the amount of damage in the field caused by the pathogens could not be determined. But experiments on durability tests on spruce timber carried out in the laboratory have shown that spruce timber is 'non-resistant' to decay caused by S. sanguinolentum and P. mitis, since this class suffers average losses in weight from 10-30 per cent during 4 months' test. There is no doubt, however, that such injuries caused by these fungi, will cause serious deterioration in the value of timber and the losses brought about by them may become more serious.

As wounds on the stem appear to be closely associated with the disease, the treatment should be preventive and not curative. Although further infection experiments on living spruce by these fungi, following pruning of living branches, are necessary, control measures based on general principles can be suggested and these may be put into effect in the affected areas in order to prevent spreading of the disease. Great care should be taken during pruning operation and thinning in order to avoid injuries caused by careless work. Pruning should be done close to the stem but not so close as to damage the bark and no projecting stubs should be left. The pruned surface should be smooth and covered with paint in order to protect the

surface from moisture and to prevent penetration of germ-tubes from germinating spores. An alternative method is to apply a fungicide to the cut surface to kill any superficial spore. Removal of all fructifications from the dead branches, as often suggested for preventing dissemination of spores, seems to be an impracticable proposition, since, tiny fructifications like those of S. sanguinolentum would invariably be overlooked during the search. Up to the present the effect on the trees of pruning has been limited almost entirely to the extent of healing process. The evidence which has now been brought forward concerning the infection by fungi as the result of wounding must now be seriously considered.



VIII. SUMMARY.

1. The present study has been undertaken to provide information which would assist in evaluating the importance of a serious disease of Norway spruce growing in a mixed stand in Glentress forest, near Peebles, in Scotland.
2. Two fungi, viz., Stereum sanguinolentum and Pleurotus mitis have been found to be the cause of the disease. They have been isolated from the internal rots of the same or different standing trees. Of these, S. sanguinolentum appeared more commonly during isolation.
3. The external and internal symptoms of the disease have been fully described, the essential features being pronounced fluting of the stem as a result of longitudinal depressions in the bark and its subsequent cracking with copious outflow of resin. The infected lesions are mostly restricted within the limits of pruning and occur round about the pruning wounds where strips of cambium have been killed due to the entry of the pathogens.
4. The isolates of S. sanguinolentum and P. mitis have been grown in culture. Their characteristic appearance and constant behaviour have been presented in potato-dextrose agar and malt agar. On these two media both the fungi behave very similarly as a rule. In all isolations of each fungus the

cultures have started in the same way but the growth rates have been greater on potato-dextrose agar than on malt agar. On the last named medium the daily rate of growth of S. sanguinolentum at 23°C in darkness has been 7.5 m.m. Under identical conditions, the daily increment of growth of P. mitis on malt agar has been 7.5 m.m. and that on potato-dextrose agar has varied between 4.5 and 7 m.m.

5. Stereum sanguinolentum and Pleurotus mitis are both white rot fungi since they produce oxidation rings on Bavendamm's medium and bleach the violet colour of the malt agar medium containing 0.007 per cent Gentian violet. This bleaching is probably due to the production by the white rot fungi, of an extra-cellular oxidase system. On tannic acid medium the intensity of reaction has been intense to very intense in both cases.
6. Typical fructifications of S. sanguinolentum and P. mitis bearing normal basidia and basidiospores have been obtained in artificial cultures originating from the infected tissues of Norway spruce. Various devices adopted to induce the cultures to fructify have been fully described. A low range of temperature (5°C - 15°C) has been found to be essential for the production of sporophores of P. mitis, below or above which fruit-bodies are not formed at all. S. sanguinolentum, on the other hand, can fructify well under ordinary conditions of

light and temperature of the laboratory.

7. Fructifications of both S. sanguinolentum and P. mitis, that developed on sections of infected trees after felling, have been thoroughly described and their anatomical peculiarities noted.
8. Microscopic characters of the rots have been fully described. The rotted tissues show the presence of mycelium in all stages of decay, appearing most abundantly in the advanced stages. It is particularly plentiful in the medullary rays which seem to be the first element to be attacked. Closely interwoven mycelium often fills up many of the ray parenchyma cells, sometimes tracheids and associated with gum-like material giving the appearance of a zone-line. The mycelium ramified at first through the pits but later penetrates the cell-walls forming numerous bore-holes. That the bore-holes result from the hyphae dissolving their way through the walls, rather than by mechanically piercing them, seems obvious owing to their smooth, molded contours and their gradual enlargement after the hyphae have penetrated the walls.
9. Chemical changes of the wood during the process of decay have been tested with micro-chemical stains in common use. The change consists mainly in the delignification of the highly lignified parts of the secondary walls of the wood-elements associated with digestion of cellulosic materials.

It is also evident that in the early stages of decay S. sanguinolentum can utilize more lignin than P. mitis while the latter digests more cellulose at the beginning.

10. The natural resistance to decay of spruce timber has been tested in the laboratory by exposing small sterilized test-pieces of the sound timber to the attack of S. sanguinolentum and P. mitis separately growing in pure culture under controlled conditions, and the amount of decay resulting from the fungal attack after 4 and 8 months tests has been calculated by estimating the loss in dry weight. After the experiments it has been found that the spruce timber is 'non-resistant' to decay since this class suffers average losses in weight from 10-30 per cent during 4 months test. It has been found that the loss in weight due to P. mitis is less than that caused by S. sanguinolentum both after 4 and 8 months' studies. But the interesting fact is that the loss in weight after 8 months is double of that after 4 months in both cases.

11. Inoculations have been made on cut twigs in the laboratory and also on trees in the field. It has been observed that when freshly cut surfaces of these twigs are inoculated with spore-suspensions of S. sanguinolentum and P. mitis in sterile water, the spores germinate readily on the surface within a short time and send down hyphae into the elements



of the wood. Since these twigs cannot be kept in an actively growing condition, their invasion by S. sanguinolentum and P. mitis cannot be regarded as an evidence for their parasitic action on trees. Nevertheless, it can be said that if by chance the spores somehow alight on the surface of the wound, they can germinate there under suitable conditions and pass down their hyphae into the elements of the wood. On the other hand, inoculation experiments on living trees carried out in the field have shown that both S. sanguinolentum and P. mitis can infect living trees through wounds in the bark, and from the results of inoculations they can be regarded as wound parasites on normal vigorous trees.

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# PLATES

PLATE I.

Fig. 1. A portion of the plantation at Glentress forest showing infected Norway spruce trees characterised by ill-defined, flat and longitudinal depressions of the bark extending from the butt upwards.

Fig. 2. A longitudinal crack in the depressed bark accompanied by an outflow of resin.

Fig. 3. Fructifications of both Stereum sanguinolentum and Pleurotus mitis have developed on respective rots at the cross-section-end of the infected pole under experimental conditions.

Fig. 4. Fructifications of Stereum sanguinolentum have developed within a couple of months at the cross-section-end of a freshly felled trunk of Norway spruce lying on the floor of the forest.





1



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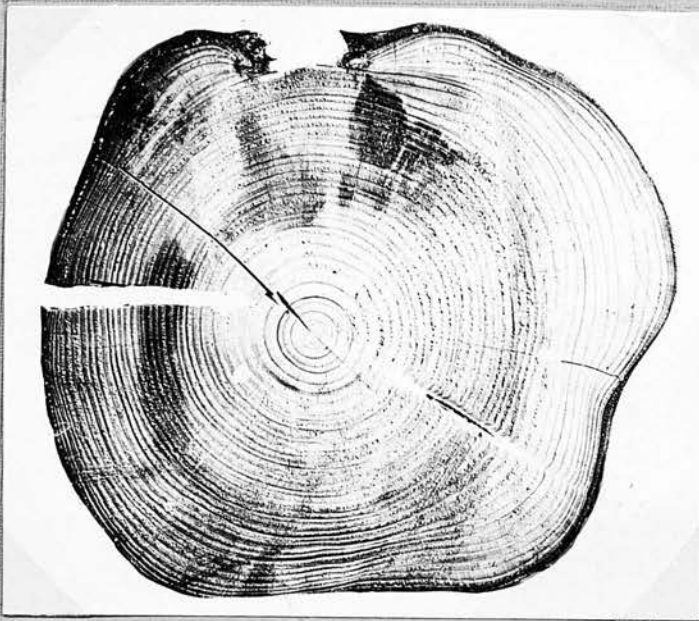
4

PLATE II.

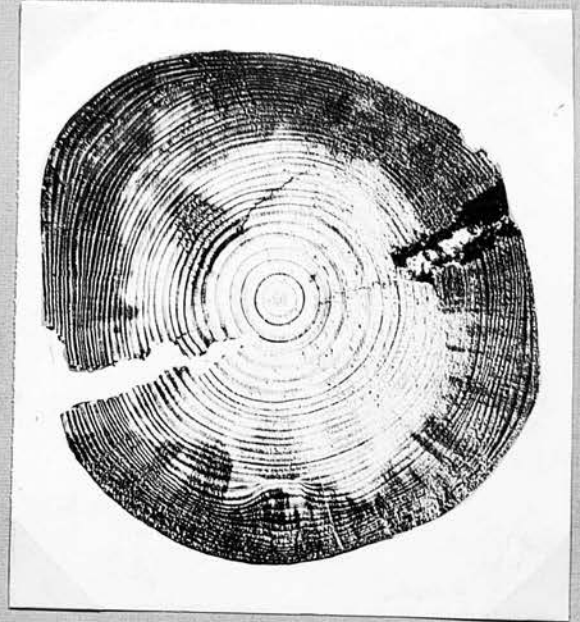
Figs. 5 - 8. Cross sections of Norway spruce poles showing various stages of decay; characteristic light to dark irregular or wedge-shaped areas can be seen from the periphery to the centre of the mature wood.

Fig. 9. Longitudinal section of the wood showing the rotted areas.

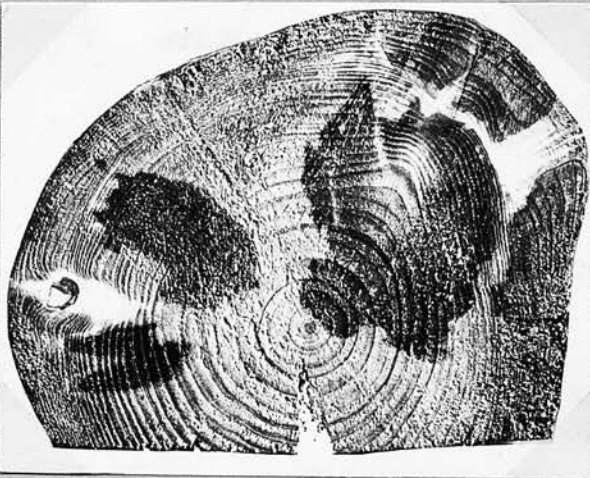
Fig. 10. A longitudinal crack in the bark with copious outflow of resin.



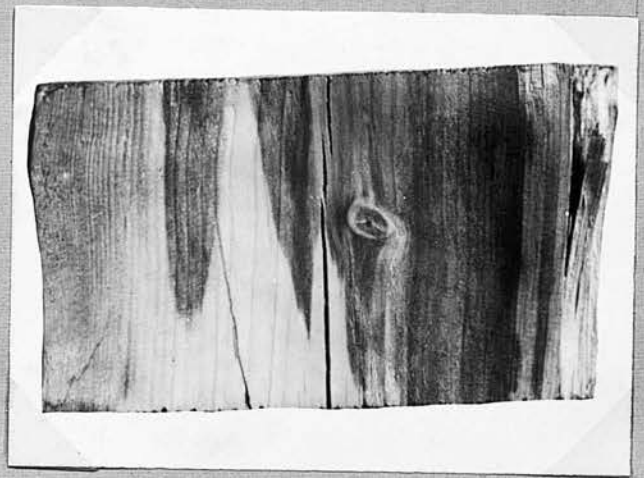
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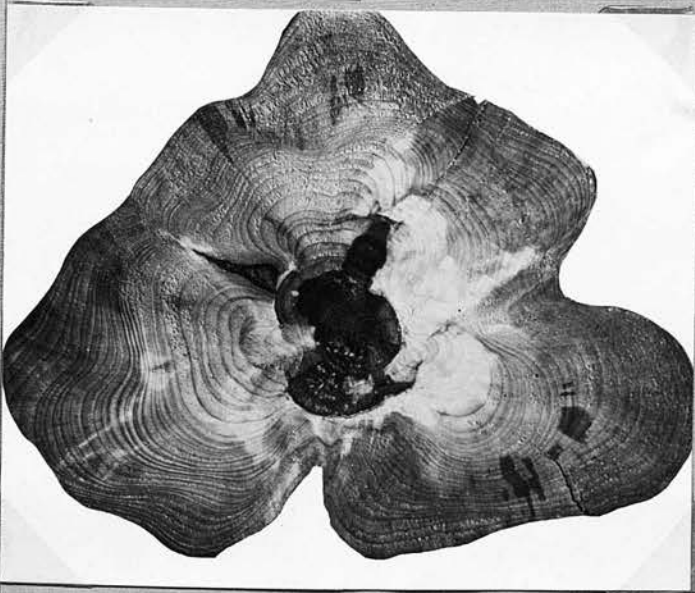
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PLATE III.

Figs. 11 & 12. Effuso-reflexed fructifications of Stereum sanguinolentum have developed on the rotted areas at the cross-section-ends of Norway spruce poles.

Fig. 13. Fructifications of Pleurotus mitis growing on a dead branch of Scots pine.

Figs. 14 & 15. Typical fructifications of Pleurotus mitis on dead twigs of Sitka spruce showing various stages of development; fig. 14, the upper surface; fig. 15, the lower surface showing the gills.

Fig. 16. Fructifications of Pleurotus mitis with abnormally elongated stipes and mycelial strands formed within the cracks of Norway spruce poles.





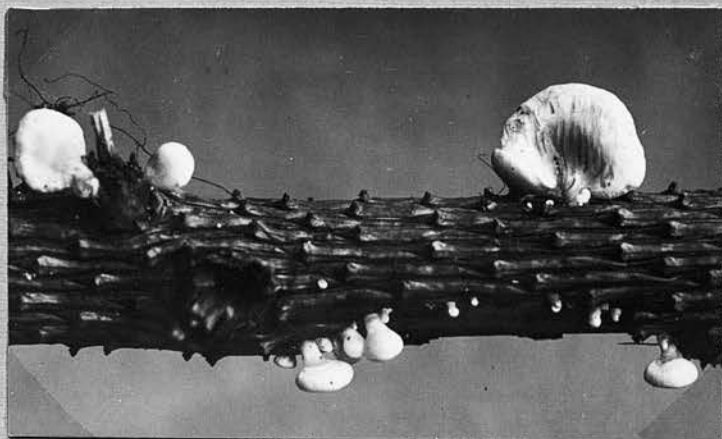
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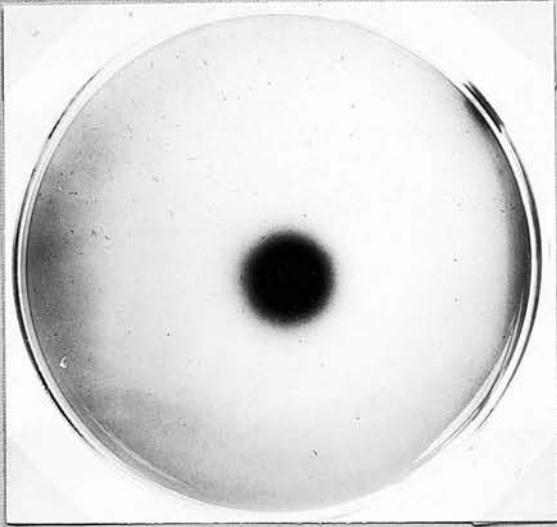
PLATE IV.

Fig. 17. Stereum sanguinolentum showing strong oxidase reaction after 24 hours on malt agar containing 0.5% gallic acid.

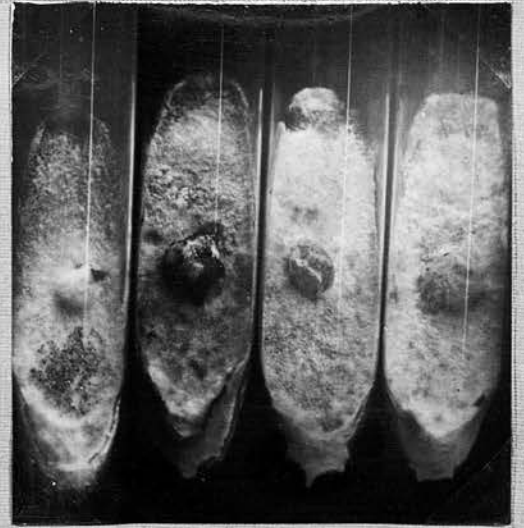
Fig. 18. Cultures of Stereum sanguinolentum 21-days-old, on potato-dextrose agar and malt agar in darkness and at 18°C (a, c) and 23°C (b, d) respectively.

Figs. 19 & 20. Cultures of Stereum sanguinolentum , 14 & 21-days-old, on malt agar at 23°C in darkness.

Fig. 21. Culture of Stereum sanguinolentum, 21-days-old, on potato-dextrose agar at 23°C in darkness.



17



*a b e d*

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PLATE V.

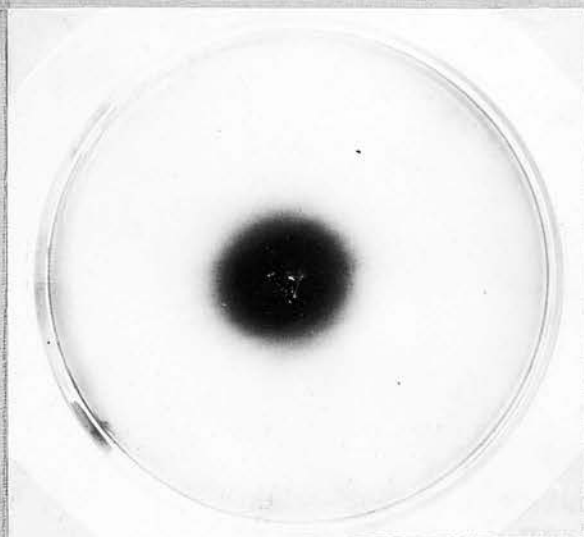
Fig. 22. Pleurotus mitis showing a very strong oxidase reaction after 2 $\frac{1}{2}$  hours on malt agar containing 0.5% gallic acid.

Fig. 23. Cultures of Pleurotus mitis, 21-days-old, on potato-dextrose agar and malt agar in darkness and at 18°C (a,c) and 23°C (b,d) respectively.

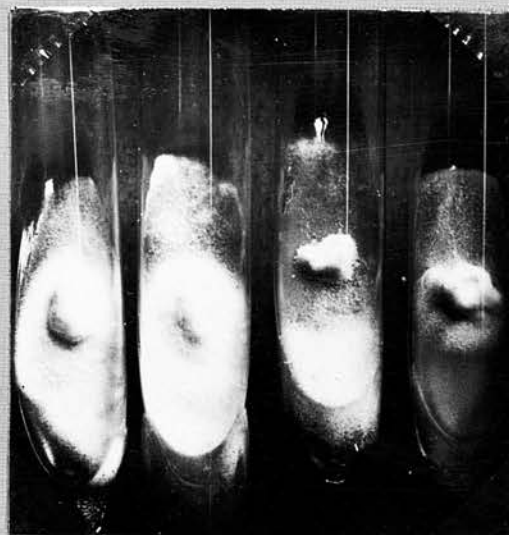
Fig. 24 & 25. Cultures of Pleurotus mitis, 1 $\frac{1}{2}$  - and 21-days-old, on potato-dextrose agar at 23°C in darkness.



PLATE V

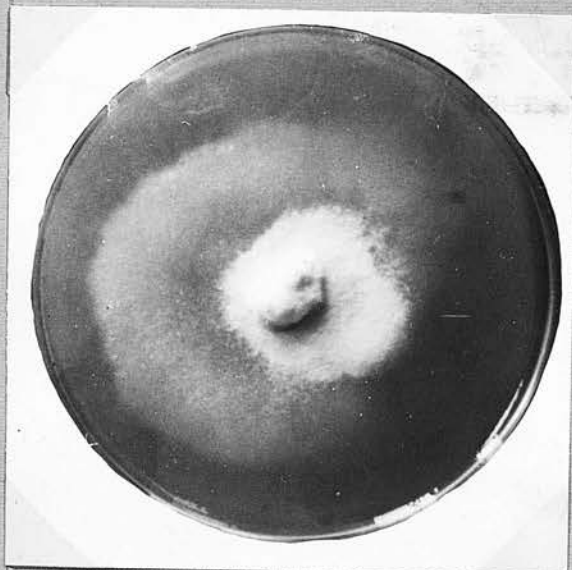


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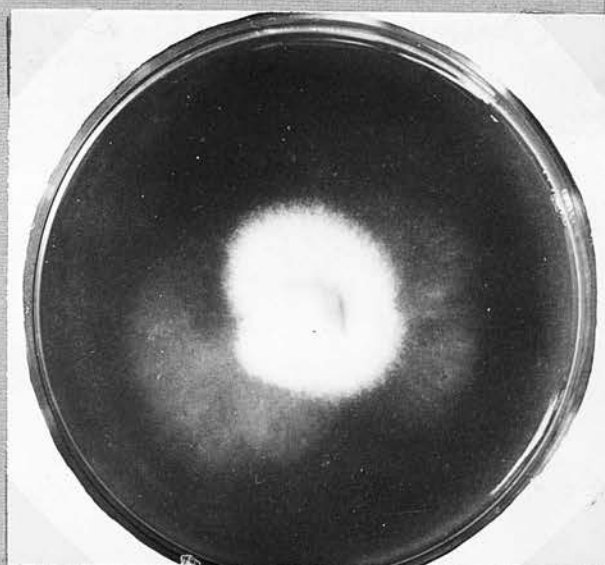


*a b c d*

23



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PLATE VI.

Figs. 26 & 27. Helicoid hyphae of the aerial mycelium of Stereum sanguinolentum (x 400).

Figs. 28 & 29. Aerial hyphae with double clamp-connections of Stereum sanguinolentum in culture (x 900).

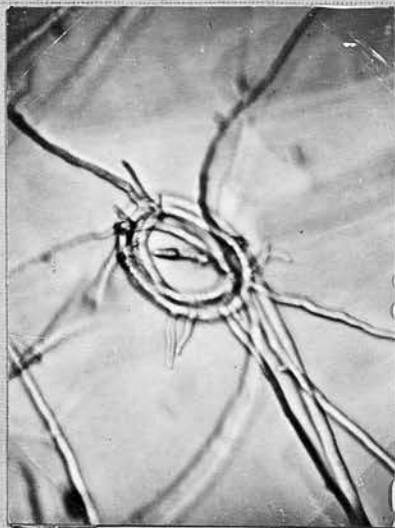
Fig. 30. Specialized, usually unbranched, flexuous hyphae with densely granular contents; characteristic of the submerged mycelium of Pleurotus mitis.

Fig. 31. Part of the cross section of a fructification of Stereum sanguinolentum showing lactiferous cells in the hymenium (x 400).

Fig. 32. Part of the radial section of the infected wood showing masses of hyphae of Stereum sanguinolentum within the horizontal resin-canal (x 100).



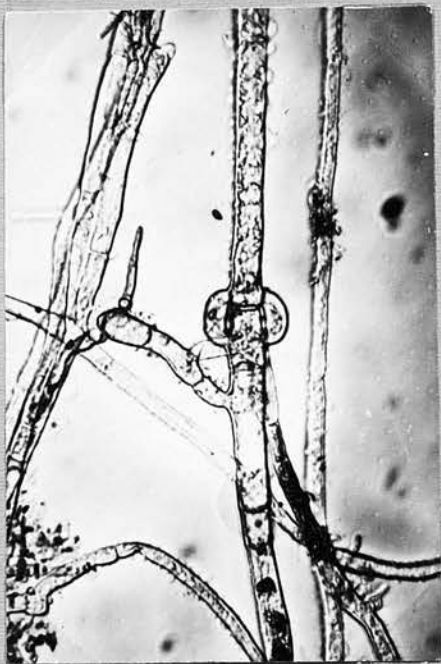
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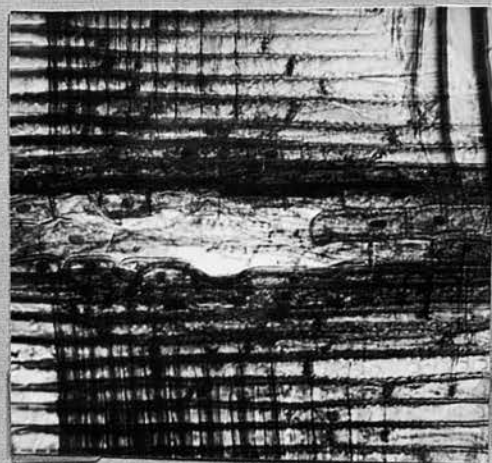
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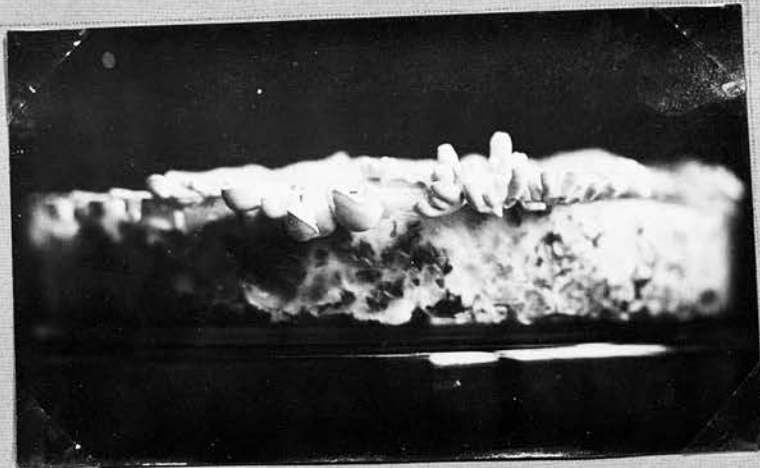
PLATE VII.

- Fig. 33. Effuso-reflexed fructifications of Stereum sanguinolentum formed within test-tubes in artificial culture.
- Fig. 34. A resupinate fructification of Stereum sanguinolentum formed on wood blocks of Norway spruce in culture.
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- Fig. 36. Fructifications of Pleurotus mitis in culture (x 4).
- Fig. 37. Mycelial strands of Pleurotus mitis developed on the surface of the infected pole under moist conditions.

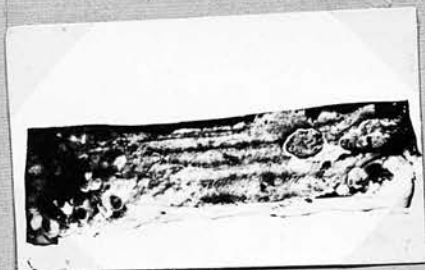




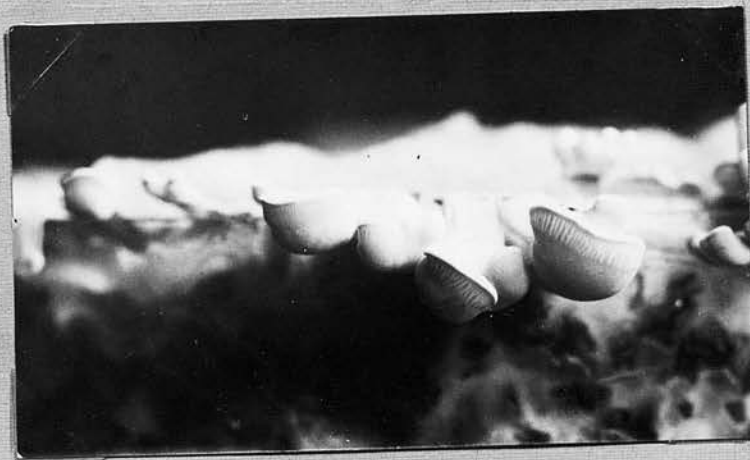
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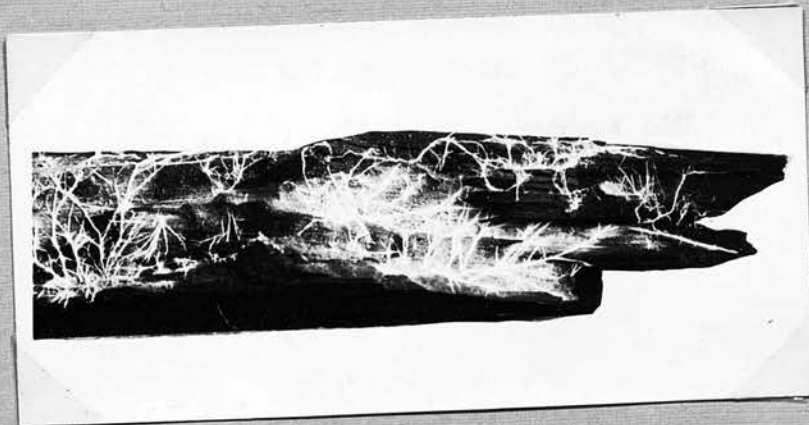
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PLATE VIII.

Fig. 38. Eroded bordered pits of the tracheids due to the activity of Pleurotus mitis; in some the border of the pit has been completely dissolved by enzymatic activity. In the pits in the middle the structure of the membrane is shown clearly as a result of the chemical action of the fungal enzymes. (x 900).

Fig. 39. Distribution of the hyphae of Pleurotus mitis within the section of decayed wood; the hyphae have ramified through the bordered pits and also through the walls forming bore-holes. (x 450).

Fig. 40. Hyphae of Pleurotus mitis within the tracheid showing simple clamp-connections; the bore-holes can be clearly seen. (x 900).

Fig. 41. Distribution of the hyphae of Stereum sanguinolentum within the sections of decayed wood; the hyphae are abundant within the tracheids and they have passed mainly through the bordered pits. (x 200).

PLATE VIII



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PLATE IX.

Fig. 42. A hypha with simple clamp-connection of Stereum sanguinolentum within the tracheid. (x 450).

Figs. 43 & 44. Hyphae with double clamp-connections of Stereum sanguinolentum within the tracheids. (x 450).

Fig. 45. Penetration of the cell-walls by the hyphae of Stereum sanguinolentum; the bore-hole is visibly larger than the penetrating hypha. (x 900).

Fig. 46. Penetration of the cell-walls by wider hyphae of Stereum sanguinolentum; the wider hypha or its branch when passing through the cell-walls, becomes markedly constricted. (x 450).





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PLATE X.

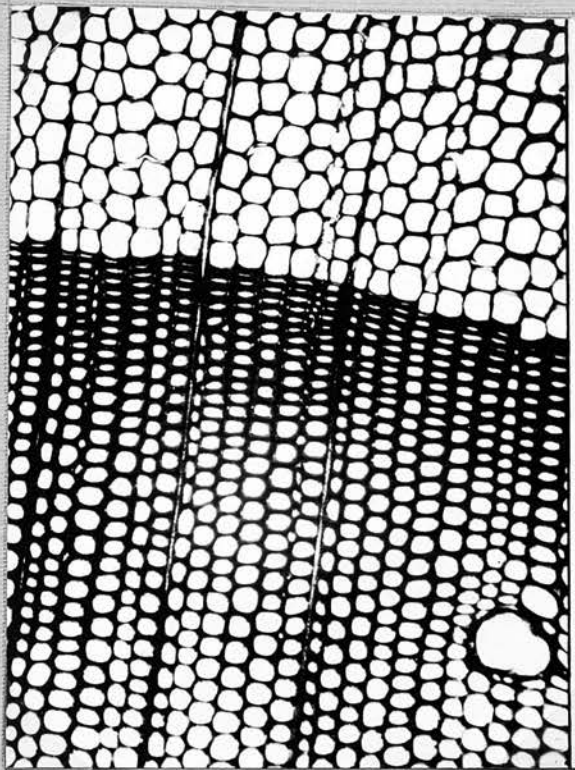
Fig. 47. Part of a transverse section of the normal wood of Norway spruce. (x 100).

Fig. 48. Part of a transverse section showing decay of Norway spruce caused by Stereum sanguinolentum; the elements of the Springwood are badly damaged. (x 100).

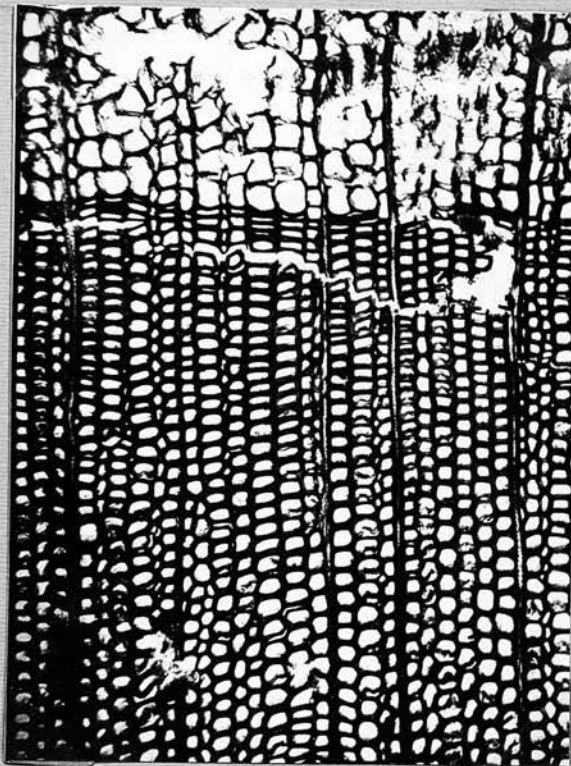
Fig. 49. Part of a transverse section showing decay of Norway spruce caused by Pleurotus mitis; the springwood is the most affected part of the section. (x 100).

Fig. 50. Hyphae of Stereum sanguinolentum passing through the walls of the bordered pits. (x 900).

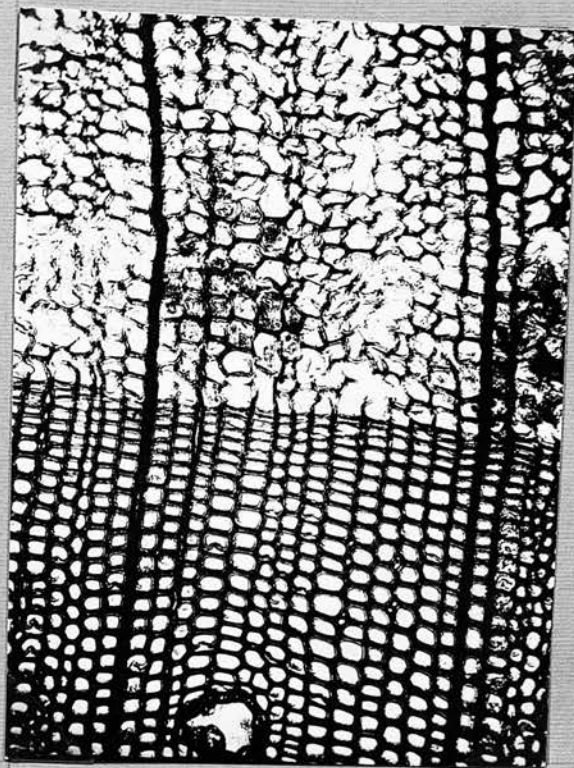
PLATE X



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PLATE XI.

Figs. 51 & 52. Wood-block cultures of Stereum sanguinolentum and Pleurotus mitis for testing decay resistance in the laboratory; photographed after 45 and 60 days respectively.

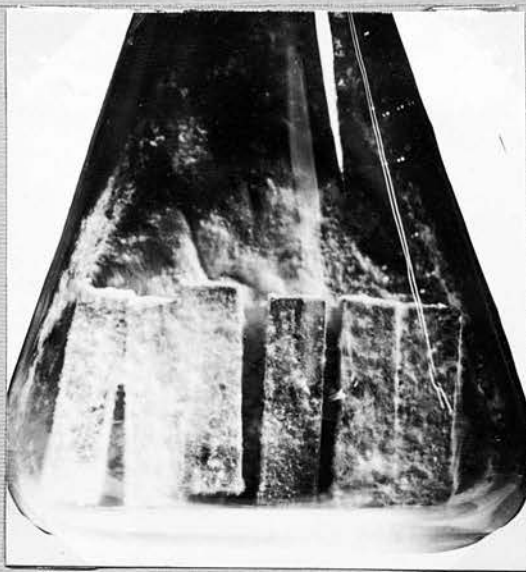
Figs. 53 & 54. Wood-blocks of Norway spruce showing decay after 4 months' exposure to Stereum sanguinolentum and Pleurotus mitis respectively.



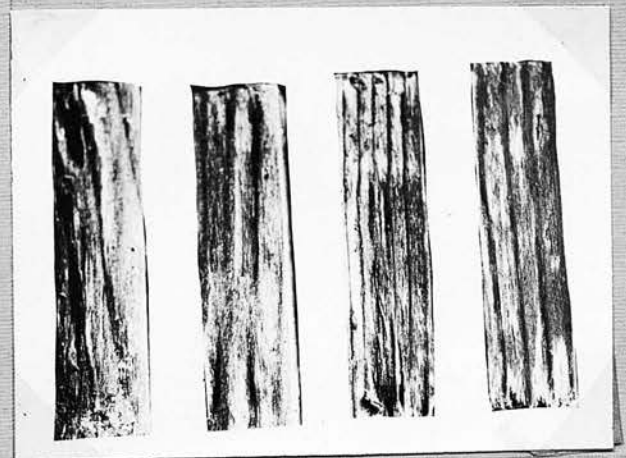
PLATE XI



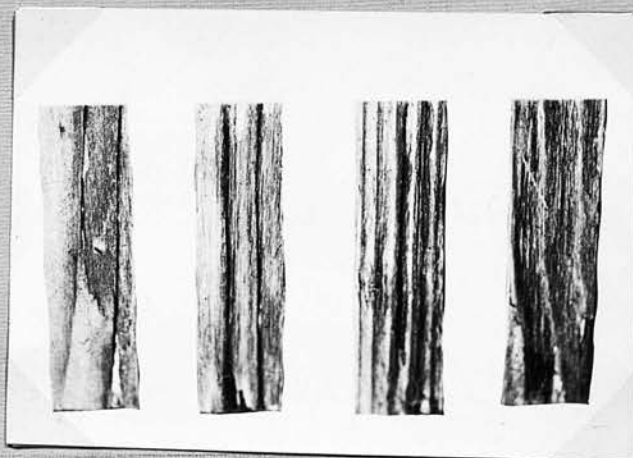
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PLATE XII

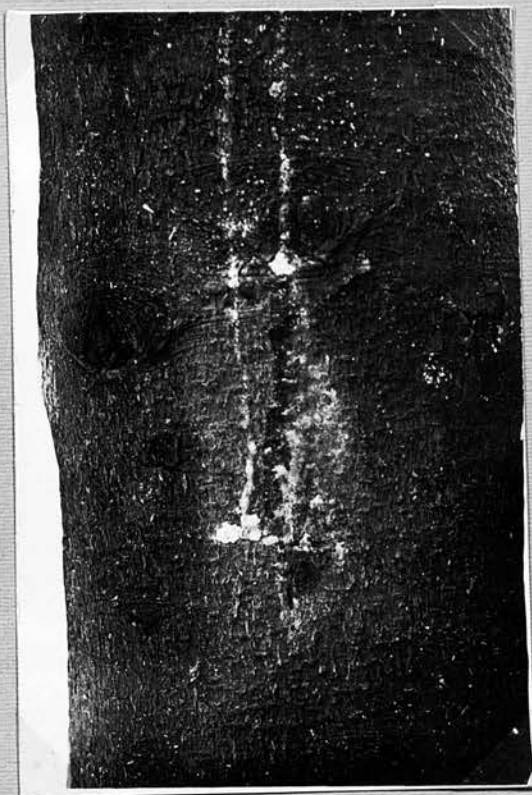
Fig. 55. Inoculated trunk of healthy Norway spruce showing infection after 5 months; the wound is gaping open accompanied by copious secretion of resin running down the stem.

Figs. 56 & 57. Similar infections on trunk of Norway spruce with Stereum sanguinolentum and Pleurotus mitis respectively, 13 months after inoculation.

Fig. 58. Longitudinal section through area of infection by Stereum sanguinolentum, showing the rot near the periphery, extending downwards from the point of inoculation.



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Plate XIII.

Fig. 59. Longitudinal section  
through the area of infection  
by Pleurotus mitis, showing  
the rot near the periphery.





II. Studies on the biology of  
Auricularia auricula-Judae (Linn.)  
Schroet. (= Hirneola auricula-Judae  
(Linn.) Berk.) causing rot in  
elder (Sambucus nigra L.)

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INTRODUCTION.

Auricularia auricula - Judae (Linn.) Schroet., the common Jew's ear fungus, is fairly distributed throughout the world and is found in both temperate and tropical regions. In Great Britain it is known to occur commonly on elders but more rarely on beech, elm, oak, walnut, willow, holly and Berberis arcuata (Rea, 1922). Besides Sambucus nigra, Poetsch (1877) lists a number of other plants, viz., Ailanthus glandulosa, Elaeagnus angustifolia, Morus nigra and Robinia pseudacacia on which it occurs. In India, it occurs commonly on logs of Shorea robusta and dead stumps and branches of Ficus religiosa (Banerjee, 1947). Secretan (1833) divided the fungus into three varieties of which var. B caraganae (Pers. Syn. p. 625) was found growing on a large dead branch of Lycium barbarum while other two varieties were always found on Sambucus nigra. Its fructifications are abundantly formed during the cold, wet months of November to February. It is often found in great quantity growing entirely saprophytically on trunks and branches without attacking the living trees but it is sometimes supposed to grow parasitically on elders (Ainsworth and Bisby, 1945). Thumen (1876) found it in autumn on two new substrata viz., Acer Negundo and Hibiscus syriacus and mentioned that in both cases the fungus grew luxuriantly on the



healthy host plant. Tunstall (1923, 1925, 1940) in Cachar, Assam (India), described it as the commonest wound parasite killing the branches of the tea plants and in time extending even to the roots, but he did not give any experimental evidence in support of his statement. Geneause and Kuenzel (1939) isolated it from the rotted heartwood of three living black walnut (Juglans nigra) trees but its etiological complications had not been established. Though a great deal of research work has been carried out on this fungus, its activities as a parasite on elder (S. nigra) and the amount of damage it causes in the wood, are not yet completely understood. With this end in view, an investigation into the nature of the disease, the amount of damage in the wood caused by it and the life-history of the fungus in culture have been undertaken. In order to establish the absolute connection between one of the higher fungi and the ultimate effects which the mycelium produces on living trees, it is imperative that an investigation of this nature must necessarily extend through several years due to the slow growth of the pathogen. In the following pages data so far obtained, are given largely from observations in the laboratory as well as in the field.

## II. THE ELDER: ITS ECONOMIC IMPORTANCE.

The common elder tree (Sambucus nigra L.), the natural host of the Jew's ear fungus, is widely distributed in Great Britain, central and southern Europe, thriving mostly in wasteland and in shady places in woods on many kinds of soil. Until recently, foresters and farmers regarded it as a plant of little value and as such it was usually heavily cut down and destroyed by burning by the landowners. Although the flowers, fruits and bark of elder have been long recognised in medicine for curing various troubles, the plant has now practically lost its reputation as having considerable therapeutic value. In the country districts of Great Britain, the berries are commonly used to manufacture elderberry wine. Recently, Metcalfe (1948) has pointed out the economic importance of elder and showed that its wood is particularly suitable for the preparation of watchmakers' pegwood and high grades of charcoal. The sticks of elder pith are usually used in Botanical laboratories for holding small specimens. Watch and other instrument makers use it for manipulating small objects. It was only during the last World War, the preparation and marketing of elder pith were

undertaken in Great Britain, as it was thought to be unwise to ignore the potential uses for any raw material available in this country. He further advocates the cultivation of elder for manufacture of pith, high grades of charcoal and timber suitable for making delicate objects and this might be undertaken profitably as a small scale industry.

### III. HISTORY AND SYNONYMY.

Auricularia auricula-Judae, the Jew's ear or Judas' ear, was well known and described at least as far back as the end of the sixteenth century. Gerarde (1597, 1633) mentioned and figured this fungus as "Fungus Sambucinus, sine Auricula Iudae, Iewes eares". Later, Micheli (1728) described and figured the fungus as Agaricum Auriculae forma. It was mentioned by Linnaeus (1753) as Tremella Auricularia. Persoon (1801) described the fungus under the name Tremella Auricula-Judae but Fries (1822-23) transferred it to the genus Exidia, under the tribe Auricularae and described it as Exidia Auricula-Judae. Berkeley (1860) redescribed the fungus under the generic name Hirneola by which it was known for a long time. Schroeter (1889) transferred it to Auricularia and this appellation had come into common use (Rea, 1922; Ainsworth and Bisby, 1945). Because of its wide spread distribution, its ability to grow on many kinds of decayed wood and its great variety of shapes, sizes and colours depending on its age or the dryness or moistness of the surrounding atmosphere, it has been described from time to time as new species under various names as the following list of synonyms indicates.

Barrett (1910) records the following:-



Tremella Auricularia L. Sp. Pl. 1157, 1753.

Peziza Auricula L. Syst. Nat. ed. 12, 2:725, 1767.

Merulius auricula Roth. Germ. I: 535, 1788.

Peziza Auricula-Judae Bull. Champ. I: 241, 1791.

Tremella Auricula-Judae Pers. Obs. Myc. 2:93,  
1799.

Auricula sambucina Mart. Fl. Crypt. Erl. 459,  
1817.

Exidia Auricula-Judae Fr. Syst. 2:221, 1822.

Auricula ampla Pers. in Freyc. Voy. 177, 1826.

Exidia auricula Wallr. Fl. Crypt. 2:559, 1833.

Exidia ampla Lév. Ann. sc. Nat. Bot. III.  
5:159, 1846.

Hirneola auricula-Judae Berk. Outl. 289, 1860.

Hirneola ampla Sacc. Syll. 6:765, 1888.

Auricularia Auricula-Judae Schröt. Krypt. Fl:  
Schles. 3:386, 1889.

Auricula ampla Kuntze. Rev. Gen. 2:844, 1891.

Auricula Auricula (L) Underwood. Mem. Torrey  
Club. 12:15, 1902.

She also considers that in all probability Auricularia auriformis (Schw.) Earle is the same fungus and if it proves to be the same, several more synonyms should be added to the list.

According to Moller (1895) both Laschia delicata Fr. and L. tremellosa Fr. are synonyms of A. auricula-Judae. He found that it is common in Brazil.

IV. THE SPOROPHORE

(Plate I, figs. 1 &amp; 2)

Description.

Fructifications. - Sessile and attached by a point or sub-stipitate; erumpent; single or in groups; often imbricate; at first cup-shaped then becoming irregularly lobed or folded, sometimes auriform or conchiform; soft, gelatinous or cartilagenous, semi-transparent, trembling when moist; dimension variable, about 1.5 - 9 x 1 - 5 c.m. when fresh; margin at first entire but somewhat lobed in older specimens, curling over the hymenium on loosing moisture.

Upper surface. - When young smooth, becoming tomentose with fine, short, sub-bulbous hairs with age; colour varies from Hay's russet to Liver brown with greyish hairs which change to Olive brown, Benzo brown, Cinnamon drab or Fawn colour on drying, finally becoming Fuscous; irregularly veined and becoming wrinkled on loosing moisture.

Context. - Gelatinous or cartilagenous, becoming hard and horny on drying; at first whitish; about 1-2.5 m.m. thick.

Hymenial surface. - At first smooth, then venoso-plicate with age; colour varied, Hay's russet or grayish, Fawn colour to Army brown, often with a lilac tinge, becoming Fuscous or Fuscous black on drying, shining or dull with a whitish bloom.

Basidia. - Elongated, cylindrical to somewhat fusiform, often slightly flexuous, transversely septate, forming a compact hymenium; sterigmata four of unequal lengths, each developing from a cell of the basidium; dimension about 55-85(100)x 5-7-10  $\mu$ .

Spores. - Hyaline; thick-walled; smooth; oblong, cylindrical or curved; dimension about 17 - 22 x 7 - 10  $\mu$ .

Tissue differentiation. - In a cross section of a mature fructification, the following regions can be differentiated:- (1) a compact hymenial layer, about 80-100  $\mu$  thick, consisting of elongated basidia; <sup>(Pl. II, fig. 7).</sup> (2) an intermediate hyaline zone making up the major portion of the context; about 1000 - 2000 (2500)  $\mu$  thick when moist; consisting mainly of hyaline, much branched, stainable hyphae embedded in a matrix formed of hyaline, non-stainable, gelatinised hyphae, mostly 1-1.5  $\mu$  sometimes up to 2.5  $\mu$  across, with numerous simple clamp-connections, running more or less parallel but becoming closely

interwoven below the hymenium. forming a brownish zone, about  $100\ \mu$  wide; (3) an outer dark brownish compact zone of densely interwoven hyphae from which the hairs arise; variable in thickness; numerous dark brown, thick-walled, flexuous or contorted, isolated upright hyphae, about  $7.5 - 10\ (12)\ \mu$  wide present; hairs thick walled, septate, sub-bulbous, hyaline to pale yellow, pointed, rounded or truncated at the tip, about  $5-8\ \mu$  wide.

The above description is based on materials collected from Hopetoun, near Edinburgh, Scotland, while growing luxuriantly on trunks and branches of living elder (Sambucus nigra). The colours mentioned in the description and afterwards are according to Ridgeway (1912). The fruiting body in itself is very characteristic and readily recognised in the field. The imbricate habit of growth, the soft, gelatinous or cartilaginous, semi-transparent, irregularly lobed, sometimes conchiform or auriform and sessile fruting bodies are distinctive features. When young and in a moist condition, it is frequently turbin-shaped but with age, in a rainy weather, it is greatly extended in size, becomes lobed and somewhat undulated at the margin and the lobes lying over one another.



## V. DECAY OF ELDER.

### A. Material.

The materials for study were collected in February 1950, in the form of freshly cut infected trees of elder (S. nigra) with fructifications of Auricularia auricula-Judae from elder bushes in Hopetoun Estate, near Edinburgh, Scotland. The situation was somewhat shady, cool and damp and as such the fructifications of the fungus were found growing luxuriantly on decaying trunks and older branches of living trees bearing numerous young upright shoots. The trees studied in this connection were carefully selected, cut down and macroscopically examined in the field to note as far as possible the progress of decay in the wood. For further study, small sections from different regions of the diseased trunk and branches were selected, brought into the laboratory, examined and studied while in a fresh condition. Microscopic examinations of the rotted areas showed abundant mycelium within the tissues of the host.

### B. Macroscopic characters of the rot.

The symptoms that are externally visible are the presence of numerous fructifications (Pl. I, fig. 1) of the fungus bursting open through the decayed or

cracked bark of the trunk and rotting of the peripheral portions of the wood where the decay has advanced to a considerable extent. The bark of the decaying wood separates easily and often patches of whitish mycelium are present in between the bark and the decayed wood. With the exception of the green shoots the fructifications are found all over the trunk. Cross sections of the infected wood show that the early stages of attack are marked by the formation of small, incipient rot pockets at the priphery of the wood being bounded internally by a narrow, water-soaked, light yellowish or pale brownish invasion zone. (Pl. II, fig. 3). These gradually become broader and lighter in colour as the attack proceeds and may finally coalasce so that in an advanced stage of decay both sap-wood and heartwood become light, soft, spongy and easily break up into sections. The bark comes away easily. In an advanced stage of decay, a cross-section of the diseased trunk shows a hollow central region (Pl. II, figs. 4 & 5) surrounded by the partially decayed wood which is somewhat whitish and crumbling. The rots showing various stages of decay appear as brownish or pale coloured irregular areas (Pl. II, fig. 4), with small pockets which may or may not contain white material and in others these are reduced to a mass of whitish or pale coloured fibres. Finally, the

wood crumbles easily and thin sheets of conspicuous whitish mycelium are found in the shrinkage cracks formed along the grains of the wood (Pl. II fig. 6). Eventually, the rotten wood falls into small flakes. The rot is confined to the trunk and older branches and does not extend into the young shoots.

### C. MICROSCOPIC DETAILS OF THE ROT.

In order to study the character and distribution of the mycelium in the infected wood, small pieces of sound and diseased wood were sectioned both free hand and with the microtome. The materials were first softened with equal parts of alcohol, glycerine and water and then transverse, radial and tangential sections, 15-18  $\mu$  thick, were cut. Free-hand-sections were, in general, found to be sufficiently thin to show the presence of hyphae in the tissue-elements but for detailed examination and successful photomicrography under high magnifications sections prepared with the microtome were particularly suitable to answer the requirements.

Differential staining was made of the sections by the methods described by Hubert (1922) and Cartwright (1929). Of these, Cartwright's safranin and picro-aniline blue were found to be most positive and satisfactory in rendering the hyphae visible and

easy to manipulate. Since this technique has got certain objectionable features, such as the hyphae often becomes contracted and distorted and the precipitation of the granular masses of aniline blue on the hyphae due to over-heating, the modified method of Proctor (1941) by using dilute concentrations and reduced amount of heat, was found to be very effective. The sections were ultimately dehydrated and mounted in balsam.

The distribution of the hyphae in the decayed wood is fairly uniform (Pl. III figs. 9-11). At first the hyphae develop mainly in the medullary rays and to a certain extent in the vessels; in the wood fibres they are comparatively less numerous. The hyphae, which are all hyaline, vary greatly in thickness, some are wide with granular contents ( $2.5 - 3.5 \mu$  across) and others quite narrow ( $1-2 \mu$  wide). The vessels are often filled up at places (Pl. III, figs. 9, 10; Pl. IV, fig. 12), with a web of wider hyphae which are sparingly branched and with frequent simple clamp-connections. (Pl. IV, figs. 14). In the narrower hyphae the clamp-connections are less numerous. The hyphae at first pass from cell to cell mainly through simple pits of the thick-walled ray cells (Pl. V fig. 16), and simple or inconspicuously bordered pits of the elements of the vessels (Pl. IV, figs. 13-15); later they directly penetrate the cell-walls by



fine thread-like pegs or outgrowths, at length enlarging the bore-holes (Pl. V, figs. 17-19). In an advanced stage of decay the hyphae become numerous and tend to become more abundant in the medullary rays and in the wider lumina of the irregularly arranged, moderately thick-walled wood-fibres with rather infrequent, slit-shaped pits which are apparently simple. Much branched hyphae often completely fill up the lumina of the fibres (Pl. III, fig. 11). The penetration of the walls of the wood cells by the hyphae either shows no diminution in hyphal diameter (Pl. V, figs. 17, 18) or in case of wider hyphae their apices become attenuated into fine points (Pl. V, fig. 19); during penetration the narrow hyphae are often clearly visible within the wider bore-holes (Pl. V, fig. 19) through which they pass. After penetration the hyphae thicken till they reach their mature width while the portion within the bore-holes remains exceedingly fine. The bore-holes, round or oval, become very numerous and are about 1-2  $\mu$  across (Pl. V, figs. 17-19). Sometimes they coalesce to form an irregularly shaped hole. The bore-holes have smooth, moulded contours and are not irregularly ruptured or splintered. This indicates clearly that the dissolution has been caused by a solvent originating from a central point. This unmistakable stamp of chemical dissolution of the cell-wall by enzymic activity of the fungus

supports Procter's (1941) theory of cell-wall penetration. At places, the bordered pits are eroded and become somewhat indistinct and transparent. The fibres become more conspicuous with spiral shrinkage cracks which radiate from the bore-holes and pits (Pl. IV, fig. 12 ). Similar spirally orientated cracks are sometimes found on the disintegrating walls of the vessels. Yellowish-brown staining of the cell-walls may develop in the vessels and medullary rays in an advanced stage of decay. A yellowish gum-like material is also present in the decayed wood.

#### D. MICROCHEMICAL STUDIES.

Results of micro-chemical staining for lignin and cellulose in the sound and partly decayed wood are recorded here. This has been done by staining the sections of wood with phloroglucin - HCl for lignin and chlor-zinc-iodine for cellulose. The combination stain, Bismarck Brown and Gentian

Violet which differentiates unlignified (brown staining) and lignified (violet staining) structures, has also been tried. Various other micro-chemical stains have also been employed, most of which agree closely with the changes indicated by the use of the above-mentioned stains. Although these micro-chemical stains are not infallible indicators of

the chemical changes that take place in the wood, yet these are commonly used in such work to indicate the presence of lignin and cellulose and the results are tabulated below.

Table 1. Results of staining for cellulose and lignin in normal and partially decayed wood of elder with Bismack brown and Gentian violet respectively.

Elements.	Normal Wood	Decayed Wood.
Vessels.	Primary walls deep violet; secondary walls partly light violet but mostly yellowish brown around the lumen.	Primary walls violet; secondary walls mostly light yellowish-brown.
Fibres.	Primary walls deep violet; secondary walls mostly light violet, deeper near the middle lamelle, mostly yellowish brown around the lumen.	Primary walls violet; secondary walls mostly pale violet but partly brownish, often deep brown at places.
Ray cells.	Primary walls violet; secondary walls mostly light violet but partly yellowish.	Primary walls mostly light violet but light brown at places; secondary walls mostly pale brown or brown but occasionally pale violet.
Wood parenchyma.	Primary walls deep violet; secondary walls light violet but partly yellowish.	Primary walls light violet; secondary walls partly light brown at places.

Table 2. Normal and partially decayed wood of elder treated with chlor-zinc-iodine and phloroghicin - HCl for staining cellulose and lignin respectively.

Elements		Cellulose (Chlor-zinc-iodine)
	Normal Wood	Decayed wood
Vessels	Secondary walls often faint blue around the lumen	Secondary walls faint blue.
Fibres	Secondary walls faint blue particularly around the lumen, often partly so.	Secondary walls partly or mostly light blue, occasionally in groups partly or entirely blue.
Ray Cells	Secondary walls partly faint blue.	Secondary walls mostly light blue or blue, primary walls light blue at places.
Wood parenchyma.	Secondary walls partly faint blue.	Secondary walls pale blue at places.

Lignin/



Table 2 continued.....

Elements	Lignin (Phloroglucin - HCl)	
	Normal Wood	Decayed Wood
Vessels	Primary walls red; secondary walls light red or pale pink to almost colourless.	Primary walls red to pink; secondary walls pink to colourless when breakdown.
Fibres	Primary Walls deep red; secondary walls light red, often pale pink near the lumen.	Primary walls red; secondary walls light red but some- what colourless near the lumen.
Ray Cells.	Primary walls deep red; secondary walls red to light red.	Primary walls red; secondary walls light red to pink.
Wood parenchyma.	Primary Walls, deep red; secondary walls red.	Primary walls red; secondary walls light red.

From the tables 1 and 2 it will be evident that phloroglucin-HCl and Gentian violet indicate the presence of lignin in the primary and secondary walls of all the elements in the normal wood. Chlor-zinc-iodine and Bismarck brown stain the secondary walls blue and yellowish brown respectively indicating the presence of cellulosic materials in the wood elements. In the partially decayed wood the chemical changes indicated by the stain-reactions clearly show that primarily there is gradual delignification of the highly lignified parts of the secondary walls and as such the residual cellulosic materials show more intense staining reaction. The reactions do not indicate clearly the removal of cellulosic materials in the early stages of decay in the wood. It can, however, be said that the fungus attacks lignin more intensely than cellulose.

Sections of normal and decayed wood corresponding to those used for micro-chemical stains have been placed on slides and treated with 72% sulphuric acid as suggested by Ritter (1925). Immediately in the sections of both normal and decayed wood, the cells swell considerably and there is much distortion throughout the tissue-elements. The middle-lamellae are torn apart, the elements are isolated in groups and the medullary rays become much convoluted in appearance. This violent

action during the process indicates the presence of considerable amount of cellulose in the cell-walls of both normal and decayed wood. The swelling of this cellulose preceding dissolution forces all the elements apart in the wood. Though not conclusive in itself, this test at least indicates the presence of much cellulose in the wood partially decayed by the fungus.

## VI. CULTURE STUDIES

### (a) Spore and Tissue Cultures.

The initial cultures were made by the use of spores and from portions of the infected wood. Copious spore-deposits were obtained within a few hours from a fresh sporophore on 2.5% malt agar contained in the lower lid of a Petri-dish, while a trimmed rectangular piece of the sporophore was fixed exentrically with the hymenial surface downwards to the inner side of its upper lid, and placed inside a moist chamber. The spores, thus deposited, were transferred aseptically to test tubes containing malt agar. The cultures were also made from partially decayed wood. Small bits of infected wood were washed several times with sterile distilled water and flamed lightly in order to kill as many superficial spores as possible from the surface. A sterile scalpel was used to cut away the exterior portions and bits of wood pieces thus exposed were carefully inserted in malt agar tubes. These were then kept in an incubator at 23°C in darkness. In the course of a week or so mycelia grew out and these served as stock materials for sub-cultures.

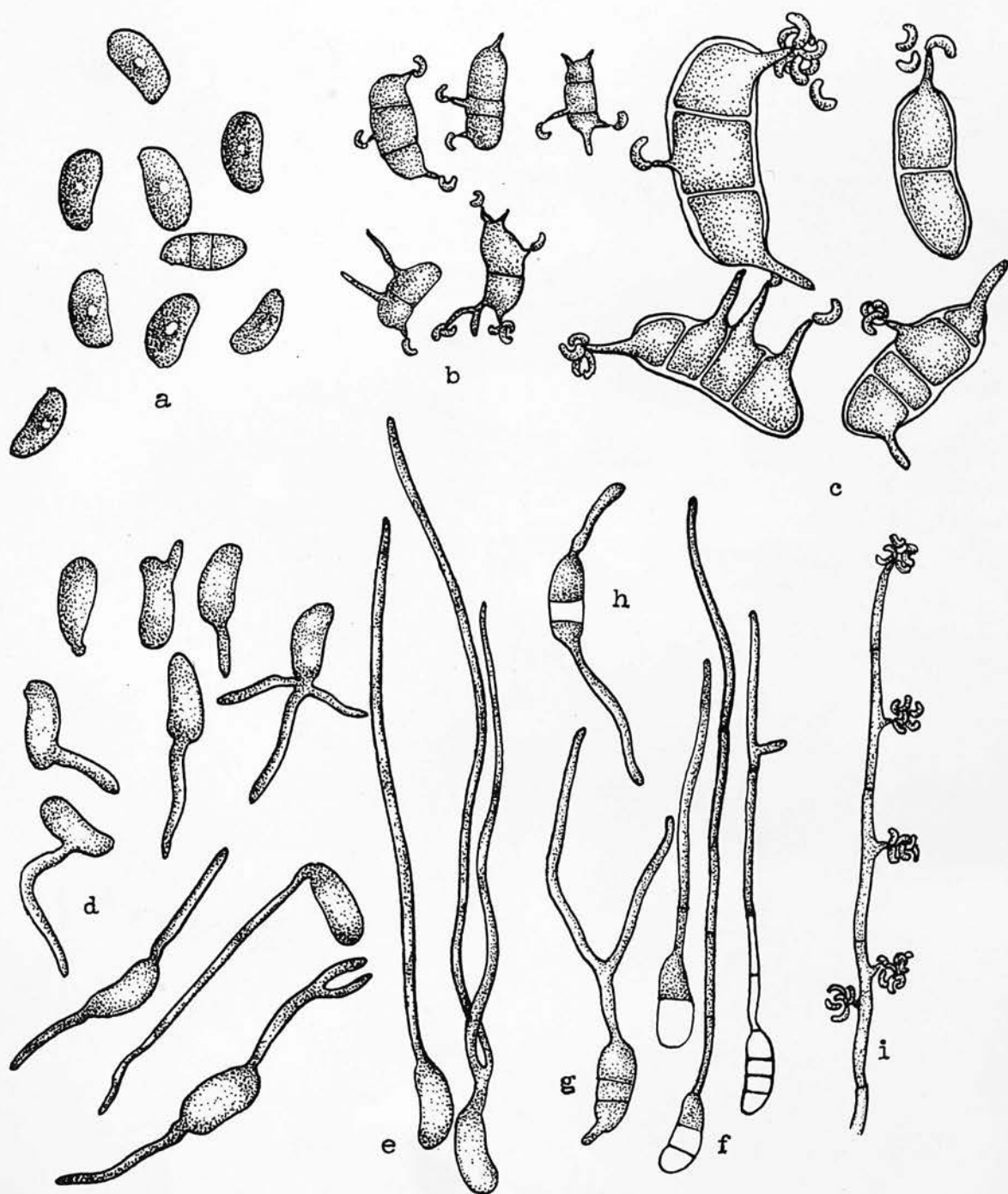
### (b) Spore germination. (Plate VIII, figs. 33-35)

The basidiospores were obtained from fresh



sporophores found on elder. The fruiting bodies continued to cast their spores for 48-72 hours and spore-deposits were obtained on sterile slides contained in a Petri-dish. Germination tests were made with these spores in hanging drop of water in Van Teigham cells and upon 2.5% of malt agar and potato-dextrose agar in Petri-dishes. Different samples of water viz., distilled water (pH7) and tap water (pH6.8) were tried. These were kept in darkness at constant temperatures of 18°C and 23°C in incubators and in diffused light and room-temperature (23°C-25°C).

On all agar media germination occurs within 12 - 18 hours under all the different conditions of light and temperature. The room-temperature and diffused light have been found to be more suitable for direct and rapid germination. The one-celled spores (Text-fig. 1,a) germinate only by producing long, thick and stout germ-tubes only either at the opposite ends or from the sides (Text-fig. 1, d,e,) When one germ-tube is produced, usually its protoplasm migrates into the tube and with its gradual advance 1, 2 or 3 septa are successively laid down within the empty spore-case (Text-fig. 1,f). This process may be continued even in the newly formed hypha where a few empty hyphal cells can be differentiated at the base from the terminal portion which is filled up with densely granular protoplasm.



Text-fig. I. *A. auricula* - Judae. Stages of spore-germination  
(x 620; c, x 1500).

A spore may form two germ-tubes (text-fig. 1,d) simultaneously at opposite ends of which one soon takes the lead and the whole protoplasm of the undivided spore retracts from the opposite side and migrates into the more actively growing germ-tube followed by successive formation of septa towards the opposite end within the original spore-wall. Sometimes the spore is at first divided by a wall into two cells, each producing a separate germ-tube. Occasionally, when two germ-tubes are produced the protoplasm spits up in the middle within the spore-case and as it moves into the germ-tubes two walls are laid down within the spore forming an empty cell at the centre (Text-fig. 1,h). Subsequent growth gives rise to luxuriant mycelium in all cases.

In water two kinds of germination have been observed. At 18°C the spores usually become divided into 2 - 4 cells from each of which one or more short or slender outgrowths are produced. These give rise to single or clusters of sickle-shaped or hoof-like conidia at the apices (Text-fig, 1 b, c) At 23°C the spores not only bear conidia from their sides but some of them germinate by producing much branched germ-tubes bearing clusters of conidia at the apices of short lateral branches (Text-fig.1, i). Occasionally, however, one or two cells of the spores

germinate by germ-tubes while others produce only conidia. Brefeld (1888) was the first to study the germination of the spores in Auricularia auricula-Judae in water and culture solution but he did not mention about the exact conditions under which the spores germinated. He stated that by using a richer and more concentrated culture solution, the spores divided rarely and produced luxuriant mycelia. In the present investigation similar results were obtained on agar media under different conditions of light and temperature and the spores also remained undivided but most often each was divided into 2 to 4 cells. In water, on the other hand, he obtained germination by the production of conidia on short side branches from each cell of the divided spore but in the present study long, much branched germ-tubes bearing clusters of conidia at the tips of short lateral branches could be obtained as a result of germination in water at 23°C. It can, however be said that low temperature and water favour germination by the production of conidia on short outgrowths from the side of the spores while in dilute culture solution and in water at a comparatively higher temperature long germ-tubes bearing conidia can be obtained. Solid nutrient media, on the other hand, inhibit the production of conidia and favour direct germination to form luxuriant mycelium under all conditions.



(c) Mycelium in culture.

The mycelium of Auricularia auricula-Judae isolated from the decayed wood as well as the polysporous mycelium were grown in culture and their characteristics studied on two different media, viz., potato-dextrose agar and 2.5% malt agar. Culture tubes 6" x  $\frac{3}{4}$ " in size were used for the study but for determining the rate of growth, they were grown in Petri-dishes. All cultures were kept in darkness in different incubators at 18°C and 23°C respectively. In order to maintain experimental conditions as constant as possible small pieces of inocula (about 3-4 m.m. in diameter) were taken from young test tube cultures and placed with upside downwards upon the medium in tubes and Petri-dishes, each containing approximately 20 c.c. and 30 c.c. of the medium respectively. The pH value of each after sterilization was found to be 5.2. The colour exhibited by the mycelium has been expressed according to Ridgeway (1912) and terms used to describe the texture of the mat are those of Long and Harsch (1918). After study it has been found that the isolate and the polyphorous mycelium are identical in cultural and microscopic details which are described in the following pages.

(1) Oxidase Tests.

The oxidase test as described by Bavendamm (1928) was made by growing the fungus in Petri-dishes on 2.5% malt agar containing 0.5 per cent gallic or tannic acid and kept at a constant temperature of 23°C in darkness. Being a white rot fungus dark brown rings due to the presence of oxidase appeared within 24 hours (Pl. VII, fig. 27 ). The intensity of reaction was, however, mild on gallic acid medium and the diameter of the ring was only 20 m.m. even after 168 hours of inoculation. Another method to distinguish white rot fungi from brown ones (Preston and McLennan 1948) was tried by growing the fungus on 2.5% malt agar containing 0.007 per cent Gentian violet. The fungus gave positive reaction and partially bleached the violet colour of the medium below the mat but about 6 weeks' incubation was necessary before definite results could be obtained.

(2) Cultural Characteristics.

Auricularia auricula-Judae (Linn.) Schroet.

(1) Habit of growth - (Pl. VII, figs. 24, 25). - On potato-dextrose agar the growth was very slow at the beginning and at the end of a week after inoculation thin cottony mycelium appeared over the inoculum with a narrow (1-2 m.m. wide) appressed growth over the

surface of the slant. In about 10-12 days the inoculum turned sub-felty to felty but the mat proper became differentiated into a central sub-felty zone and an appressed, colourless and fimbriated zone of advance. As the growth advanced the central zone remained sub-felty as before but gradually thinned out towards the upper end of the slant and became raised and cottony to silky towards the lower end. After three weeks the mycelium covered the whole surface of the slant and the mat became felty and homogeneous but with a few raised lumps of mycelium around the inoculum. The surface of the mat gradually turned tough, rough and nodulose at places. A thick skin was ultimately formed and its surface was mostly rugulose but pulverulent at places. At 23°C the growth was somewhat similar but more rapid and condensed with wider fibriated advancing zone. The texture of the mat early became cottony-woolly which later turned felty and the silky nature of the mycelium towards the lower end of the tube was more pronounced. The upper end of the mat, instead of becoming thin, was raised and cottony. Finally, tufted and felty patches of mycelium were distributed over the rugulose surface of a thick skin. On malt agar at both temperatures this habit of growth was more or less similar to that on <sup>dextrose</sup> potato agar but in the former it was more vigorous, raised, loose and woolly and the advancing zone was

less distinct. The mat proper instead of being rugulose turned pulverulent with age. In all cases colour of the medium turned yellowish in about 10-15 days after inoculation.

(ii) Colour - On potato-dextrose agar the mycelium remained white throughout but at 23°C pale salmon colour developed towards the lower end of the slant in about a month after inoculation. On malt agar pigmentation of the mat became evident in about a month and the shades included pale pinkish cinnamon and light pinkish cinnamon. At 18°C colouration was more pronounced and was restricted to the central part of the mat.

(iii) Rate of growth - The rate of growth, both on potato-dextrose agar and malt agar kept under identical conditions as stated before, was rather very slow and the daily increment in diameter of Petri-dish cultures was determined. The radial growth in all cases was unequal and as such average measurements were taken. On malt agar at both temperatures the daily increment of growth varied between 2.5 and 3 m.m. while on potato-dextrose agar it was only 2.5 m.m.

(iv) Hyphal characters - (Pl. VII, figs. 29) -

Aerial mycelium: (a) wider hyphae, about 2-3  $\mu$  wide, more or less straight or slightly flexuous, septate, distantly branched, with frequent clamp-connections,



clamps often developing into new hyphae, with yellowish brown granular contents; (b) profusely branched, hyaline to yellowish brown, narrower hyphae, about 1-2  $\mu$  across, usually highly flexuous, sometimes slightly so or more or less straight and with numerous clamp-connections.

Submerged mycelium: (a) wider hyphae, about 2.5-5  $\mu$  across, sometimes up to 7.5  $\mu$  wide due to swelling, septate, flexuous, profusely branched, irregularly swollen presenting a somewhat gnarled appearance, often constricted, with yellowish or brownish granular contents, branches often clustered, clamps present but infrequent; (b) hyaline, thin-walled narrower hyphae, about 1-1.5(2.5)  $\mu$  across, usually slightly flexuous, sometimes straight and unbranched for a considerable distance, usually sparingly branched and with clamps almost at every septum and with highly granular contents.

(d) Production of fruit-bodies in culture.

Several unsuccessful attempts were made to obtain fruit-bodies of Auricularia auricula-Judae on various agar media kept under different conditions of light, temperature and moisture. Barnett (1937) obtained fertile but atypical fruit-bodies on agar media but he did not mention anything about the exact conditions under which they were formed.

Eventually, the medium for developing sporophores recommended by Badcock (1941) was tried with considerable success. Moist sawdusts of beech or spruce with 5% Badcock's accelerator was used in Petri-dishes, test-tubes (20x4 c.m. and 15x2 c.m.) and Badcock's apparatus and the various devices recommended by him (1943) were tried. After sterilization the media were inoculated by the fungus and the cultures were kept in darkness at room temperature (18°C - 21°C) inside a closed chamber in which a moderately high humidity was maintained. Subsequently, when mycelia made a vigorous growth and covered the surface of the medium or reached the ends of the tubes, all the cultures were removed and placed in strong diffused light on a table about 8 feet from a large window of the laboratory.

Although a dense, white mycelial growth covered the entire surface of the sawdust medium in Petri-dishes, fructifications did not appear in any one of them even in cultures 3-4 months old. At this stage the lids of the Petri-dishes were removed and the cultures were kept covered under a moist bell-jar near a window at room-temperature. Within 24 hours small fructifications began to appear rapidly at the rim of the Petri-dish and they were about 8 m.m. in diameter within 48 hours. (Pl. V, fig. 21) The fructifications gradually enlarged, at first

resupinate, but later became typically reflexed (Pl. *VI*, fig. 20 ). Sections of such a well-developed fructification revealed a well-developed hymenium with normal basidia and viable basidiospores. Similar fructifications were also obtained in tubes after a period of 2-3 months. When the mycelium completely filled the tubes and became compact, the plugs were removed and each was supported in an inclined position in between two Petri-dishes, the lower being filled with water. A thick pad of absorbent cotton wool was placed within the mouth of the tube and was never allowed to dry out. Within a few days when vigorous surface growth developed, the pad was gently pushed down so as to touch the medium. Irregular fructifications began to develop within the tube and this irregularity was probably due to limited space within the mouth of the tube for their development. In Badcock's apparatus, fructifications also began to appear within the flask at the rim of the tube (Pl. *VI* fig. 23 ) after 3-4 months but their growth was very slow and somewhat irregular. This was in all probability due to limited space within the flask and possibly owing to absence of ultra-violet light or too high a humidity as pointed out by Badcock (1943).

(e) Heterothallism.

It has long been established that the absence of clamp-connections in single spore cultures and their appearance when certain single spore cultures are paired, have formed the basis for determination of heterothallism in Hymenomycetes and for the separation of bipolar and tetrapolar species. Polysporous cultures of Auricularia auricula-Judae and those obtained from the wood decayed by the fungus always revealed the presence of numerous clamp-connections and on the other hand these were completely lacking in monosporous cultures which pointed towards the heterothallic nature of the fungus. Barnett (1937) was the only investigator to describe Auricularia auricula-Judae as 'heterothallic' and sexually 'bipolar' but he did not attempt to describe the mechanism by which the nuclei of one mycelium would pass into another mycelium and form clamp-connections. He found considerable sterility between single spore cultures obtained from fruit-bodies growing on wood of coniferous and non-coniferous trees and incidentally suggested that within this species, several or many sexual phases might exist, only two such phases being so far recorded in the same fruit-body. In order to find out the type of sexuality existing in this strain of Auricularia auricula-Judae, ten monosporous cultures were



started from spores gathered from a single sporophore growing on living trunk of Sambucus nigra. The method of obtaining monosporous cultures was somewhat similar to that suggested by Mounce (1929). A portion of the fruit-body was fixed excentrically to the inner side of the cover of a filtered malt agar plate, kept under a moist bell-jar and examined under a microscope at intervals. When the spores began to drop vigorously, the cover was removed and placed over another agar plate, quickly rotated for 2-3 seconds and in this way the spores were distributed. After about 24 hours, well-germinated spores were located under the microscope, isolated by punching out small discs of agar containing single spores and transferred to 2.5% malt agar in tubes. Similarly, a spore-suspension in sterile distilled water was spread on an agar plate; germinating spores were isolated the following day and grown in culture. After growing for several days they were thoroughly examined and found to be without clamp-connections. The monosporous mycelia were then paired in all combinations on agar slants in test tubes where they were allowed to grow for more than a month. The line of contact between the paired mycelia was then carefully examined for the presence of clamp-connections. The mycelia were said to be compatible when clamps were found in the two mycelia and any irregular results obtained

		AB			ab				aB	Ab	
		1	2	10	3	4	8	9	5	6	7
AB	1	-	-	-	+	+	+	+	-	-	-
	2	-	-	-	+	+	+	+	-	-	-
	10	-	-	-	+	+	+	+	-	-	-
ab	3	+	+	+	-	-	-	-	-	-	-
	4	+	+	+	-	-	-	-	-	-	-
	8	+	+	+	-	-	-	-	-	-	-
	9	+	+	+	-	-	-	-	-	-	-
aB	5	-	-	-	-	-	-	-	-	+	+
Ab	6	-	-	-	-	-	-	-	+	-	-
	7	-	-	-	-	-	-	-	+	-	-

TABLE III.

from pairings were repeated. The results of this examination are recorded in table 3. The (+) sign indicates the presence of clamp-connections and (-) sign indicates their absence.

From the table it is evident that this strain is hetrothalllic and quadrisexual and hence markedly different from the bipolar saprophytic strains so far recorded. The cultures fell into four groups based upon the production of clamp-connections in certain combinations. The four sexual phases are designated as AB, aB, Ab and ab. No clamp-connection was, however, found between the members of the same sexual phase. Whitehouse (1949) states that instances are not uncommon where species have been recorded as bipolar by one person and tetrapolar by another and therefore it has been assumed that each of these taxonomic species is really a complex of at least two natural species and they have been recorded as such. Further statement will not be made at the present moment pending a more detailed investigation with this strain now in progress. It is interesting to note that in this strain the spores are much larger than those belonging to the strains studied by Barnett (1937).

Although no work has been done on the phenomenon of aversion, it was undoubtedly present in a number of cases. In all the pairings three types were observed, viz., (1) a space of aversion of variable

width, (2) slight aversion and the narrow gap was partially filled with dicaryotic mycelium, and (3) complete intermingling of the mycelia. (Pl. VIII figs. 30, 31)



VII. NUCLEAR PHENOMENA OF SPORE-  
GERMINATION AND IN THE MYCELIUM.

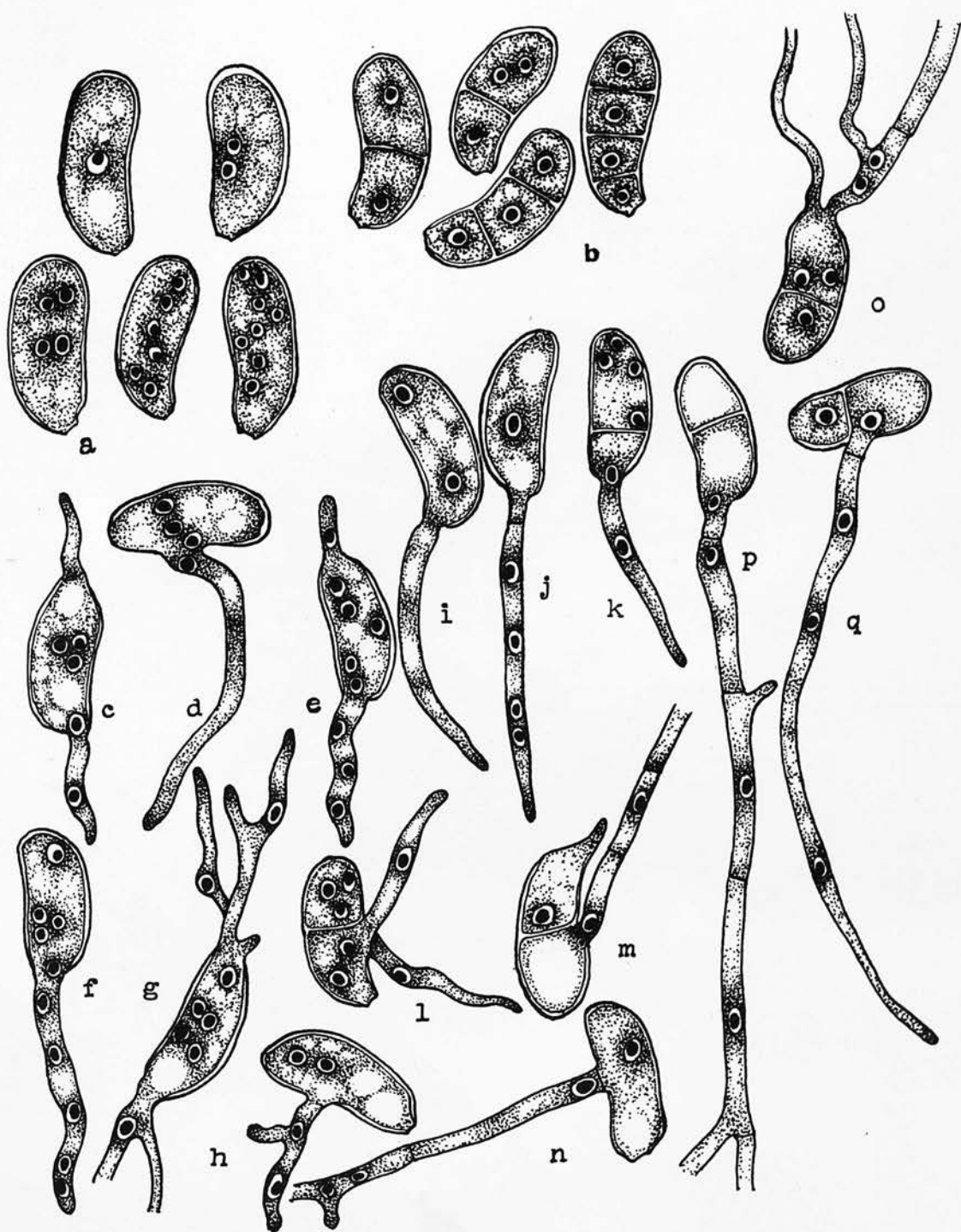
Incidentally, the nuclear phenomena of spore-germination and in the vegetative mycelium have been studied. The information so far available is scanty. Brefeld (1888) germinated basidiospores in dilute culture solutions and obtained a luxuriant mycelium on which he did not find any clamp-connection. Sappin-Trouffy (1896-97) who was the first to study nuclear details in the basidium of Auricularia auricula-Judae, incidentally mentioned that the fructification of the fungus was composed of hyphae with binucleate cells, the nuclei being reduced to specks of chromatin. Green (1925), while reporting the occurrence of clamp-connections in the fructifications of Hirneola auricula-Judae stated that owing to the presence of deeply staining granules the nuclei and their relationship to clamp-connections could not be ascertained with certainty. An attempt has, therefore, been made to study the nuclear phenomena of spore-germination and in vegetative mycelium of Auricularia auricula-Judae and a preliminary account has been presented in the following pages.

In this study, Kniep's (1913) agar film technique and that modified by Sass (1929) were mainly followed. In order to avoid undue external contamination special rectangular chambers as those

recommended by Noble (1937) for hanging drop culture but large enough to hold microscope slides on which the agar film was borne (Pl. VIII fig. 32). The composition of the medium was however, slightly modified as follows: malt extract 2 per cent, gelatin 1.5 per cent, agar 1.5 per cent and distilled water 1000 c.c. The constituents were dissolved in distilled water, filtered through a filter paper in a steamer, tubed and sterilized in Koch sterilizer at 100°C. When required, the medium was liquified by heating in a water-bath, a few drops of liquid agar were placed on a warm sterile slide and spread as a thin film, taking care that the film did not reach the edges of the slide. As soon as the agar-films had solidified they were inoculated with the mycelium, placed in an inverted position on rectangular chambers as stated above and these were kept inside sterile Petri-dishes, each containing a moist filter paper from which a rectangular piece had been cut out at the centre for allowing light to be transmitted during examination. For obtaining germinating spores and haploid mycelia, spores were directly dropped on the agar film from a piece of fresh sporophore. For early stages of spore-germination very thin films were used in order to obtain hyphae growing more or less in the same plane but for growing and pairing monosporous mycelia to produce dicaryotic mycelium, a comparatively thicker

film was used so that the medium might not be used up quickly during mycelial growth. All these cultures were incubated at 23°C in darkness and examined when necessary under sterile conditions. When the cultures had reached the desired conditions, the slides were transferred to dry Petri-dishes and the films were allowed to dry down at the margin before killing and fixing were done. Several killing and fixing fluids were tried such as Flemming's fluid (weak), Bouin's fluid (Allen's modification) and that recommended by Sass (1929). The visual results varied with the type of fixing fluid used but Flemming's fluid yielded the most satisfactory result. For early stages of germination, Sass' fluid was, however, found to be unsatisfactory and led to the bursting of the tips of young hyphae. Various stains were tried but Flemming's triple and Heidenhain's iron-alum-haematoxylin with light green as counter-stain proved to be satisfactory. Of the two, Flemming's triple stain was particularly suitable for studying nuclei in the early stages of spore-germination.

The nuclei of the spores have been stained readily and counted. It has been found that mature spores are mostly uninucleate although occasionally 2 to 3 nuclei have been observed. (Text-fig. II, a ). This may be interpreted as due to unusual division of the pre-existing nucleus of the spore. When



Text-fig. II. *A. auricula* - Judae. Nuclear phenomena of spore-germination and in primary mycelium. (x 1400).

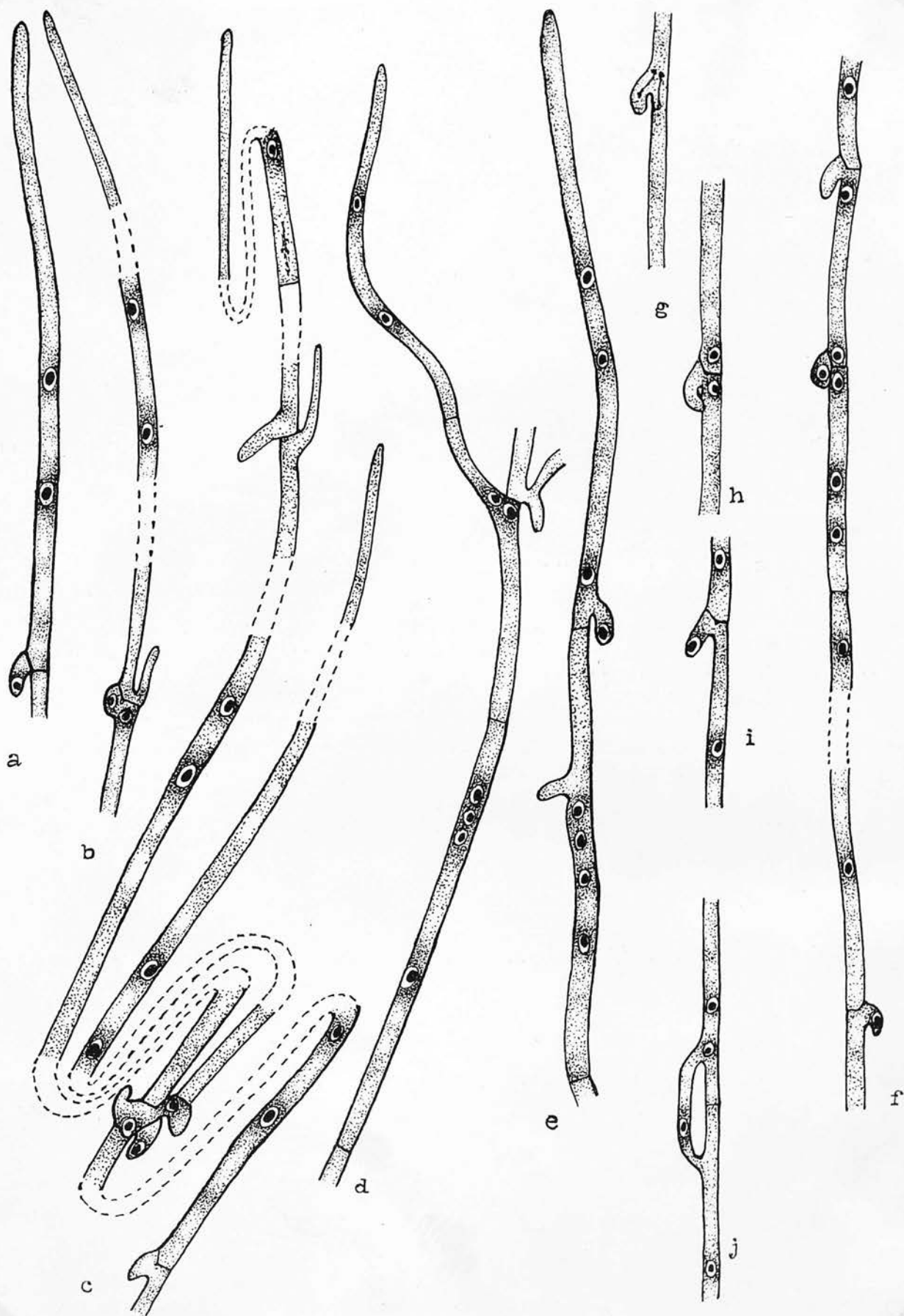


germination starts this nucleus either divides into 2 to 4 nuclei and transverse walls are laid down between them to form 2 to 4 uninucleate cells (Text-fig. 2,b) or their number may increase by repeated division and up to 8 nuclei have been counted (Text-fig. 2,a). In the latter case when the germ-tube begins to develop at one or both ends of the spore, the nuclei migrate into the tube and this coenocytic condition soon passes over into a uninucleate condition by more or less delayed formation of septa (Text-fig. 2, c to j.) The largest number of nuclei which has been observed in an unbranched tube is 5. (Text-fig. 2,f). The end-cells are not always uninucleate but often contain two nuclei. This is possibly due to the division of the pre-existing nucleus and delay in septum-formation. It has been observed that at the beginning these cross-walls are formed independent of the nuclear phenomena and they are rather difficult to demonstrate. When, on the other hand, the spore is divided into 2 to 4 uninucleate cells, the nuclei of one or more cells may remain undivided but the nuclei of those cells which give rise to germ-tubes always divide (Text-fig. 2, k to m) and all or some of the daughter nuclei migrate into the developing germ-tubes (Text-fig. 2,o) from which subsequently uninucleate hyphal cells are produced

by the formation of transverse walls (Text-fig. II, o). The advanced primary or haploid mycelium consists of uninucleate cells and without clamp connections. (Text-fig. 2, m,p.)

The structure of the fixed and stained nucleus may be described here. It practically occupies the entire width of the hypha. (Text fig. 2, p,q.) Its nucleolus stains intensely with iron-haematoxylin and in a carefully destained preparation it can be observed clearly. Besides nucleolus there are highly stained granules within the nucleus but no indication of any reticulum has been observed. A clear space is often decernable within the nucleus <sup>between the nucleolus</sup> and the nuclear membrane. The nature of a few deeply staining granules which frequently occur within the hyphae (Green 1925) could not be determined with certainty but they are undoubtedly much smaller than the normal nuclei.

As a result of pairing between primary monosporous mycelia of opposite sexes, the secondary mycelium bearing clamp-connections has been obtained. In a properly fixed and stained preparation they are readily recognised but the actual place of origin of the secondary hyphae was obscured due to the presence of a dense web of slow growing highly anastomosed primary hyphae at the line of contact. They can be easily traced back some distance into the culture rather than the actual place of origin. For this



Text-fig.III. A.auricula - Judae. Parts of secondary mycelium showing nuclei and different types of clamp-connections. (x 1400).

reason it was not possible to study critically the details of the process of diplodization and conjugate division of the nuclei. However, the cells of the secondary mycelium are typically bi-nucleate (Text-fig. 3, a,b), although 3 to 4 nuclei with plane septa are not uncommon. (Text-fig. 3, d,e). This shows that the members of the conjugate nuclei can divide independently even when there is no question of clamp-connection. The occurrence of clamp-connection in the secondary mycelium is not, however, regular. (Text-fig. 3, c.e.) The same hypha may bear plane septa as well as clamp-connections. In the former case, this absence is possibly due to division of the nuclei in a conjugate pair independent of each other forming 3 to 4 nuclei but in the latter when simultaneous division of the two nuclei of a conjugate pair takes place, in which the two spindles are to be accommodated side by side in the narrow lumen of the hypha, a method for widening the hypha to facilitate division, is adopted resulting in the formation of clamp-connection. Although no attempt has been made at present to study in details the formation of clamp-connection and the nuclear phenomena associated with it, yet from the structural point of view three types of clamp-connections can be recognised as follows.



- (1) In the first type the form agrees closely in salient points with the descriptions given by Bensaude (1918) and Kinep (1919-20). A short slender lateral pocket grows out from the side of the terminal hyphal cell, curves downwards, touches the main hypha (Text-fig. 3, g,h.) and ultimately fuses with it. Before or after fusion one cross wall appears across each spindle of the dividing nuclei near the base of the pocket and across the hypha touching each other at an angle (Text-fig. 3,b). In this type of clamp-connection the terminal cell receives a pair of conjugate nuclei as soon as the division is complete but the sub-terminal cell and the lateral pocket (Text-fig. 3,a) are both temporarily uni-nucleate. When the lateral pocket fuses with the sub-terminal cell the latter becomes binucleate.
- (2) The second type agrees closely with the first type but the lateral pocket does not fuse with the main hypha. A gap is formed between the point of contact of the two cross-walls and the lateral pocket so that the nucleus can pass easily into the sub-terminal cell below. How this gap is formed could not be ascertained with certainty. (Text-fig. 3, f,h,i). This type of clamp-connection is very common in Auricularia auricula-Judae and although figured by Barnett (1937) has not yet been fully described.
- (3) In the third type, which is uncommon, the

lateral pocket instead of curving and fusing with the main hypha just below its place of origin, continues to grow like a small branch and after proceeding a little distance, curves and fuses with the main hypha so that when clamp is formed a wide opening is seen between it and the parent hypha (Text-fig, 3, j. )

It has been<sup>that</sup>noticed/there is no constant time relationship between nuclear division, wall-formation and the fusion of the lateral pocket excepting in that the wall-formation which always takes place after nuclear division.

VIII. DECAY RESISTANCE TESTS IN THE  
LABORATORY.

The natural resistance to decay of wood block samples of elder was tested in the laboratory. The process in general involved in exposing small sterilized samples of elder to the attack of Auricularia auricula-Judae growing in pure culture under controlled conditions and the amount of decay due to fungal attack was estimated by measuring the loss in dry weight. Various methods were suggested and durability tests on different timbers were made by previous workers like Humphrey (1916), Baxter (1925), Hubert (1929), Findlay (1938) and others but the method used by the writer was<sup>as</sup> follows. Small test-blocks were cut and planed to  $\frac{1}{2}$ " x  $\frac{1}{2}$ " square and 2" long with the long axis paralalled to the grain of the wood. In all cases clear material, free from defects were taken. To find out the actual loss from decay, the test-blocks were serially numbered on all faces with a soft lead pencil and were dried in an oven at 60°C to a constant weight. The drying of the wood to a constant weight and recording of the weight were necessary where the rate of decay was the object of experiment. The blocks were then thoroughly soaked in distilled water, put in Roux tubes with water at the bottom, plugged and sterilized at 15 lbs. pressure for 10 minutes. The

object of this was to sterilize the wood blocks in a saturated atmosphere so that little loss of water could take place from the surface of the wood blocks and consequently lesser time was needed for sterilization as pointed out by Chidester (1937, 1939). These blocks were then aseptically taken out and exposed to vigorously growing cultures of Auricularia auricula-Judae in 1000 c.c. Erlenmyer flasks, each containing about 250 c.c. of 2.5% malt agar. These flasks, each containing 6 samples of wood were kept at a constant temperature of 23°C in darkness. Within a month, all the surfaces of the wood blocks were covered with smooth, white and felty mycelium but on the surface of the medium the mycelium formed a compact regulose mat with shades of light Vinaceous cinnamon to Vinaceous cinnamon mixed with white, raised, felty areas. In order to obtain a significant loss in weight the periods of exposure to fungal attack were 4 months and 8 months as recommended by Cartwright and Findlay (1946). After completion of the tests the flasks were opened, the superficial mycelium was carefully removed without damaging the wood blocks, weighed and oven dried in a similar manner, the resulting weights compared with the original and the percentage loss on dry weight of the sound wood had been calculated (Pl. VII, figs. 26, 27).



Table 4. Decay resistance of elderwood after 4 months' test.

No. of blocks.	Initial oven-dry weight (grms.) before test.	Final oven-dry weight (grms) after test.	Loss	Loss %
1	4.053	3.729	.324	8.0
2	4.217	3.630	.587	13.9
3	4.492	3.769	.723	16.1
4	4.512	4.025	.487	10.8
5	3.964	3.465	.499	12.6
6	3.827	3.512	.315	8.2
7	3.984	3.528	.456	11.4
8	3.856	3.472	.384	10.0
9	4.092	3.414	.678	16.6
10	4.164	3.568	.596	14.3
11	3.820	3.216	.604	15.8
12	3.986	3.598	.388	9.7

Average loss in dry weight % ..... 12.3

Range - 8.0 to 16.6)

Table 5. Decay resistance of elder after 8 months' test.

No. of blocks.	Initial oven-dry weight (grms) before test.	Final oven-dry weight (grms) after test.	Loss	Loss%
1	3.987	3.125	.862	21.6
2	3.858	2.938	.920	23.8
3	3.807	2.545	1.262	33.1
4	4.015	3.079	.936	23.3
5	3.984	3.089	.895	22.5
6	3.986	2.764	1.222	30.7
7	3.818	2.596	1.222	32.0
8	3.958	3.013	.945	23.9
9	3.894	2.932	.962	24.7
10	3.999	3.204	.795	19.9
11	3.864	2.965	.899	23.3
12	3.918	2.893	1.025	26.2

Average loss in dry weight %..... 25.4

( Range - 19.9 to 33.1)

The tables 4 and 5 show that the average losses in weight of elder wood in cultures, due to Auricularia auricula-Judae, are 12.3% and 25.4% after 4 and 8 months respectively. When the respective values of the loss in weight in 4 and 8 months are compared, the loss in weight after 8 months is <sup>nearly</sup> twice of that after 4 months. This loss is quite significant as the test-pieces have lost a high percentage of their original weight during the test and it can be safely concluded that the wood has low resistance to decay. Findlay (1938) has classified the timbers which he tested, into five groups in response to their natural resistance to decay and accordingly elder wood falls under his 'non-resistant' group as this class undergoes average losses from 10-30% during 4 months' test.

The moisture contents of the wood blocks at the commencement of the experiment and 4 and 8 months later have been determined. The average moisture content of the wood blocks before they were exposed to fungal action was 98.3% while that after 4 and 8 months' tests were 94% and 83.4% respectively. No attempt has, however, been made to control the exact moisture content during the experiment, still it was necessary only to find out whether the moisture content was above the fibre-saturation-point (20-30% of the oven-dry weight of the wood) or not for the activity of the wood-rotting fungus in bringing about the loss as pointed out by Cartwright and Findlay (1945).

IX. INOCULATION EXPERIMENTS.

In the present investigation inoculation experiments were carried out with cut twigs in the laboratory and also with living trees in the field. In the former case the method followed by Brooks and Moore (1923) was followed. In this experiment young twigs of S. nigra about 4" long were kept with their ends in water, their upper ends were cut off and spore-suspensions of Auricularia auricula-Judae in sterile water were applied to the freshly exposed surface and never allowed to dry out. These were then placed under a moist bell-jar at 23°C in darkness. Longitudinal sections were made through the inoculated ends of the twigs at intervals of 24, 48, 72 and 96 hours after the spores had been added. Sections cut after 48 hours show that the majority of the spores had germinated on the surface and had sent hyphae down into the elements of the wood ultimately forming a vigorous mycelium in about 96 hours. Since these twigs could not be kept in an actively growing condition, their invasion by Auricularia auricula-Judae could not be regarded as an evidence for its parasitic action on trees. Nevertheless, it can be said that if by chance the spores somehow alight on the surface of the wound, they can germinate on the surface under suitable conditions producing germ-tubes which in some way



pass down into the elements of the wood.

Field inoculations were started on healthy living trees of Sambucus nigra and Sambucus racemosa in April 1950. The trees inoculated were either young, about 2 to 3 years-old or much older and were growing in a well-lighted open situation. In the latter case only the lower branches were inoculated. Inoculations were made at different times of the year for a period of ten months (March 1950 to January 1951). The operation involved was to make a cross incision through the bark and the branches were inoculated by transferring actively growing mycelium from tube cultures into the wound by carefully lifting a flap of the bark still attached to it. In case of very young twigs longitudinal tangential incisions were first made downwards through the outer skin in order to obtain a flap of the skin still attached at the base. The inoculum was then placed into the wound under the flap. Before inoculation the surface of the twig was wiped out with absolute alcohol and subsequently washed with sterile distilled water in order to free the surface from contamination as far as practicable. Each flap was then covered with moist absorbent sterile cotton wool with a piece of thick paper over it to prevent drying out and firmly bound with a string. Three types of inocula were

used for this experiment and these were taken from monosporous, polysporous and dicaryotic mycelia made available for the purpose. At the beginning, twelve inoculations were made, six of each, keeping adequate controls. In case of Sambucus racemosa only three inoculations were done on the skin with controls below. The string and the cotton-wool were removed after a month and the inoculated branches were left exposed in that condition. At this stage no external sign of infection was, however, visible.

After about four months the trees were again examined and it was found that, in all cases, both S. nigra and S. racemosa seemed to have taken up the infection. (Pl. IX figs.<sup>36,37</sup>). The wounds were gaping open and somewhat swollen only in case of S. nigra. The invaded wood was discoloured and somewhat dark brown in colour. The controls remained flat and there was no such discolouration of the wood below the bark. After about 8 months the infected wounds became more conspicuous and there was considerable amount of swelling around the wounds in case of S. nigra but no such swelling was observed in S. racemosa. At this time no fruiting bodies of the fungus were formed and there was no other indication of decay. This was only to be expected owing to the very slow growth of the fungus even when grown on culture media under favourable

conditions. As there were no other external effects of inoculation the internal conditions of the infected branches were examined microscopically. Mycelium was found within the tissues of all the branches inoculated. It was however, not uniformly distributed within the tissues of the host. In both cases, the hyphae were present in the bark and living cortex but especially abundant in the peripheral portion of the wood. The pith and inner layers of the wood were without any trace of hypha. In the cortex, the hypha ramified through the intercellular spaces but also found penetrating the living cells. They spread more rapidly through the vessels in a longitudinal direction than in the cortex. Penetration in the transverse direction was rather slow. Very little can be said at present as to the ultimate effects produced by the advanced mycelium and the greatest distance to which the mycelium had penetrated into the wood from the point of infection. It may, however, be said that in case of S. nigra the hyphae transversed about half an inch in 4 months and to about an inch in 8 months from the point of infection in longitudinal direction while in S. racemosa this was much less, being 8 m.m. in about 4 months. All these point to the fact that Auricularia auricula-Judae under conditions favourable to infection is able to infect elder trees. This was further confirmed by re-isolating the fungus from

different infected lesions.

Another set of experiments was started in October 1950, when young twigs of S. nigra were inoculated with monosporous mycelia. After a period of 4 months these were examined and positive results were obtained.



X. DISCUSSION.

A study on Auricularia auricula-Judae and its effects in the living trees and on sterilized wood blocks make it possible to discuss in general some of the salient points regarding the relations that exist between the fungus and the host. Fructifications of the fungus are commonly found to grow on the trunk and older branches of living elder trees (Sambucus nigra) and as such the fungus is often supposed to be a parasite. At present there exists some differences of opinion among the investigators regarding the definition of a true parasitic wood-rotting fungus. According to some, parasites are those fungi which grow upon living hosts while others regard those as parasites which actually derive nourishment from the living tissues of such hosts. Therefore, in order to determine whether a wood-rotting fungus is strictly parasitic or not, it is essential to find out the presence of living elements within the tissues invaded and whether the fungus actually attacks and destroys such cells. Microscopic examinations of the different regions of elder wood shows that the only living elements in the sap wood are the parenchyma cells filled with starch grains while the fibres and the cells constituting the vessels have already died. In the heartwood, on the other hand, the

elements are all dead and the food materials have been transferred out of the parenchyme cells during the process of maturation. It is, therefore, not imperative that a fungus attacking the sap wood would necessarily invade the living parenchyma cells, since the ultimate death and disintegration of such cells may be incidental during the process of digestion of the dead elements of the wood by the activity of the fungus.

Inoculation experiments on healthy elder trees (S. nigra and S. racemosa) with Auricularia auricula-Judae carried out in the field have shown that the fungus can invade living tissues of the cortex through wounds in the bark and the cambium is killed at the place of infection. The mycelium is both inter and intracellular. In such cases the progress of decay would be from the bark to the woody portion of the stem. The course of infection, however, depends entirely on the initial point of infection, whether on the injured bark or on wounds due to cutting down of the branches. It has also been experimentally proved that under controlled conditions the spores of Auricularia auricula-Judae are able to germinate on freshly cut surfaces of elder twigs and can send down hypha into the tissues of the host. In such cases, it is apparent, that in all probability the woody portion of the stem would be attacked first and the causal agent after entering

the host would spread from the centre to the periphery of the stem. It has not been possible to study the progress of such an infection in the field. From the results of inoculation experiments however, it can be regarded as a wound parasite killing the living cells of normal vigorous trees although the fungus shows a preference to grow in the wood deriving its <sup>food</sup> entirely from the dead wood elements and in extreme cases cause severe heart rots.

Whenever fructifications of Auricularia auricula-Judae were collected for this investigation in most cases it was found to grow upon the stub of a broken branch or the old trunk of a living tree which was almost in the process of dying. In such cases, both sap wood and heartwood were particularly decayed. In some cases the sporophores were, however, seen to burst through the bark of living older branches borne on the decaying trunk and this may be regarded as a positive case of parasitism. Microscopic examination of the branch revealed the presence of mycelium in the living tissues and in the sap-wood from which cultures were made. The bark became loose, separated easily and a mass of whitish mycelium was found in between the bark and the sap wood.

During field study it has been observed that the trees at Hopetoun, which formed the basis of

this investigation and were growing in a shady, low and moist situation, were attacked by the fungus. Trees growing wild at other places on higher land have been found to be somewhat protected from infection possibly due to lower humidity of the air which is unfavourable for spore-germination. It can therefore, be assumed that the fungus does not usually attack trees of normal vigour but it would spread if the trees were unfavourably circumstanced. It has already been stated that the foresters and landowners regard elder as a plant of little value and as such its branches are usually heavily cut down at any time of the year forming open wounds. This also has been the fate of the infected trees at Hopetoun and as such Auricularia auricula Judae being a wound parasite possibly entered through these wounds and brought about infection. The fungus is common there as a saprophyte on dead branches and during November to February forms fructifications. The spores, after being shed and carried away by the wind, alight on the cut surfaces of the branches or on wounds in the bark and bring about infection under favourable conditions prevailing there.

The amount of damage in the field caused by Auricularia auricula-Judae has not however, been determined. Experiments on durability tests on



elder samples carried out in the laboratory have shown that elder wood is 'non-resistant' to decay due to Auricularia auricula-Judae, since this class suffers average losses in weight from 10-30 per cent during 4 months' test. There is no doubt that this loss is quite significant and such injuries would cause serious deterioration in the quality of the wood.

XI. SUMMARY.

1. Auricularia auricula-Judae, because of its wide range of distribution in the temperate and tropical regions throughout the world and its adaptability to grow on a great variety of wood, is undoubtedly responsible for the deterioration of large quantities of wood annually. Although commonly found as a saprophyte, it has been found to grow as a parasite on elder (Sambucus nigra).

2. The fructifications of the fungus have been thoroughly described and their peculiarities noted. They are readily recognised in the field by their soft, gelatinous, semi-transparent, irregularly lobed, sometimes conchiform or auriform and sessile fruiting bodies, becoming hard and horny on drying.

The fruit-body is composed of hypha showing numerous clamp-connections.

3. Inoculation experiments on healthy standing trees of both S. nigra and S. racemosa carried out in the field have shown that the fungus, in both cases, can attack the living tissues through wounds in the bark but in all cases the growth of the mycelium has been very slow. The fungus, however, shows a preference to grow in the wood and in extreme cases cause severe heart rots. It has also been experimentally proved that under laboratory

conditions the spores of Auricularia auricula-Judae are able to germinate on freshly cut surfaces of young elder twigs and can send down hypha into the tissues of the host. From the results of inoculation it can be regarded as a wound parasite on normal vigorous trees.

4. For this investigation fructifications were found growing upon living trunks and comparatively older branches of elder, usually bursting open through the decayed or cracked bark. Close examination showed that the younger branches had been regularly cut down annually whereby open wounds were exposed. Through these wounds the fungus presumably had infected the trees and ultimately attacked the heartwood, thereby rendering it unfit for commercial use. These trees were growing on a low, moist situation and were found to be attacked with the fungus. Host species at other places growing on higher land were found to be protected from infection, possibly owing to lower humidity of the air and the consequent poorer condition for spore-germination.

5. In the early stages of attack the fungus produces incipient rot pockets at the periphery of the wood but in an advanced stage of decay both sap-wood and heart-wood are attacked which are rendered light, soft, spongy and easily breaking up into sections. The heartwood becomes hollow at the centre and the

rotten wood eventually falls into small flakes. Thin sheets of conspicuous whitish mycelium are found in the shrinkage cracks along the grains of the wood. The distribution of hyphae within the decayed wood is fairly uniform, being particularly abundant in the medullary rays and to a certain extent in the vessels in the early stages of decay. The clamp-connections are more frequent in the wider hyphae. Bore-holes are numerous in the advanced stage of decay and the fibres and vessels are often completely filled up with mycelial wefts associated with yellowish gum-like material.

6. Chemical changes in the wood during the process of decay have been tested with micro-chemical stains in common use. In the partially decayed wood the change consists primarily in gradual delignification of the highly lignified parts of the secondary walls of the elements and as such the residual cellulosic materials show more intense staining reaction.

7. Experiments on durability tests in the laboratory have shown that elder wood is non-resistant to decay due to Auricularia auricula-Judae since this class suffers average losses in weight from 10 - 30 percent during 4 months' test. The average losses in weight of elder wood in cultures have been 12.2% and 23.3% after 4 and 8 months respectively.



8. Cultures of the fungus have been made from spores and tissues of the decayed wood. Their characteristics have been presented on potato-dextrose agar and malt agar. In all isolations the cultures started in the same way and the average rate of growth on both the media has been slow and more or less the same, but a little faster on malt agar. It is a white rot fungus as it gives positive oxidase reactions on Bavendamm's medium.

9. Under ordinary conditions of light and temperature of the laboratory typical fructifications of Auricularia auricula-Judge bearing normal basidia and viable basidiospores have been obtained in artificial cultures originating from the infected tissues of elder. Various devices adopted to induce the cultures to fructify have been described. Cultures on various agar media, however, have not fructified at all.

10. The basidiospores have germinated readily in water and on agar media kept under various conditions of light and temperature. In water at 18°C they have germinated only by producing conidia but at 23°C many of the spores have given rise to much-branched germ-tubes bearing clusters of conidia at the tips of short lateral branches. On agar media under all conditions direct germination by producing germ-tubes has been observed.

11. The spores at first mostly uninucleate.

At the time of germination this nucleus either divides and 2-4 uninucleate cells are formed within the spore or the division is continued without wall-formation to form a multinucleate spore. The nuclei migrate into the germ-tubes and this coenocytic condition soon passes over into an uninucleate condition by more or less delayed formation of septa. These cross-walls are formed independently of the nuclear phenomena and they are rather difficult to demonstrate. Sometimes the nuclear division and wall-formation take place in succession so that from the beginning uninucleate primary mycelium is produced. The cells of the secondary mycelium are typically bi-nucleate although 3-4 nuclei with plane septa are not uncommon. The occurrence of clamp-connections is not, however, regular. The same hypha may bear plane septa as well as clamp-connections. From the structural point of view three types of clamp-connections have been described. It has been observed that there is no constant time relationship between nuclear division, wall-formation and fusion of the lateral pocket excepting in that the wall-formation which always takes place after nuclear division.

12. Auricularia auricula-Judae is heterothallic and not strictly bi-sexual. Pairings of monosporous

cultures have been made and the cultures have fallen into four groups based upon the production of clamp-connections in certain combinations. Therefore, this parasitic strain is hetrothallic and quadrisexual.

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# PLATES



PLATE I.

Fig. 1. A group of fructifications of Auricularia auricula-Judae on a dead trunk of elder (Sambucus nigra).

Fig. 2. A group of young fruit-bodies of A. auricula-Judae growing on the cut end of a stout branch; the lower portion of this branch bore living shoots.

PLATE I



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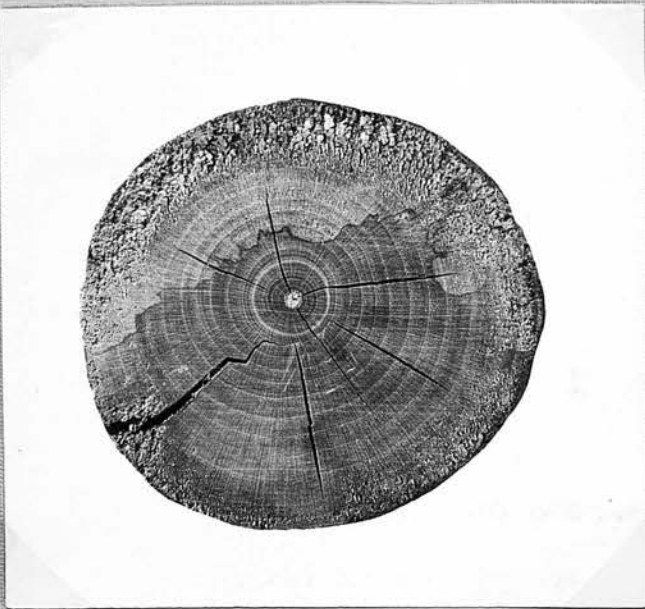
PLATE II.

Figs. 3 & 4. Cross sections of the infected wood showing various stages of decay; the brownish, pale-coloured irregular areas with small pockets can be seen.

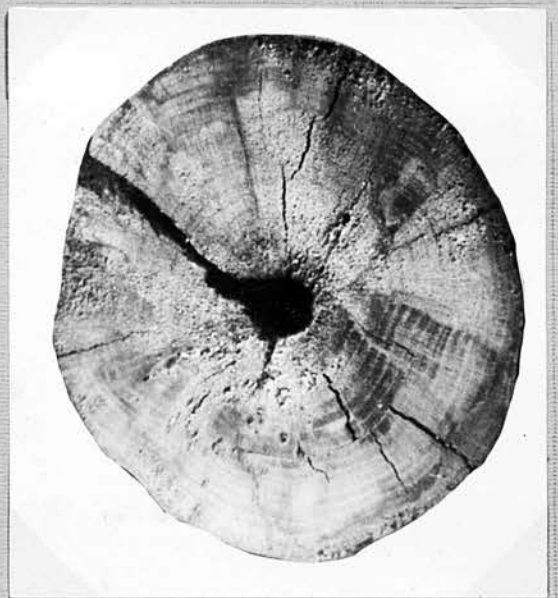
Fig. 5. Longitudinal section of the wood showing the rot; the pith has been completely destroyed.

Fig. 6. Mycelial sheets in between the shrinkage cracks formed along the grains of the wood.

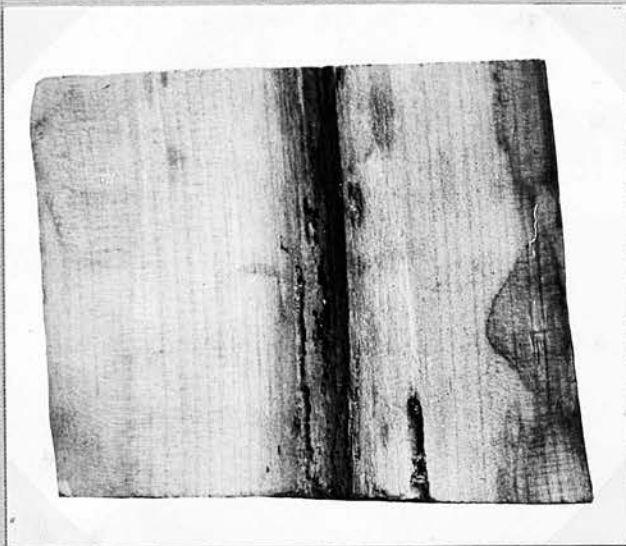
Fig. 7. A cross section through the fruit-body of Auricularia auricula-Judae showing the hymenial layer (x 200).



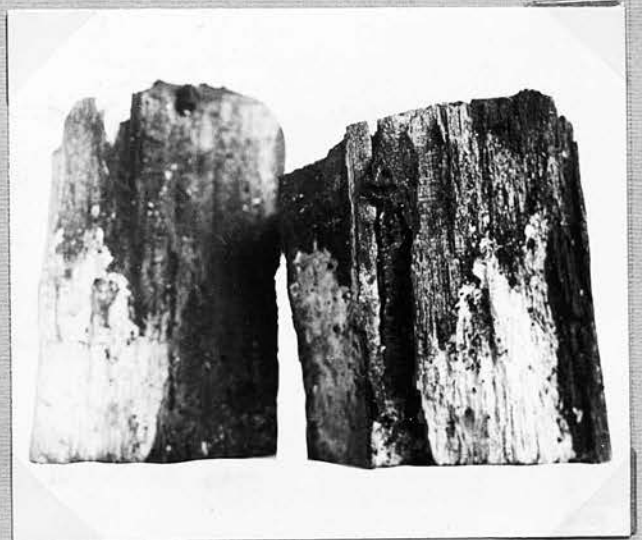
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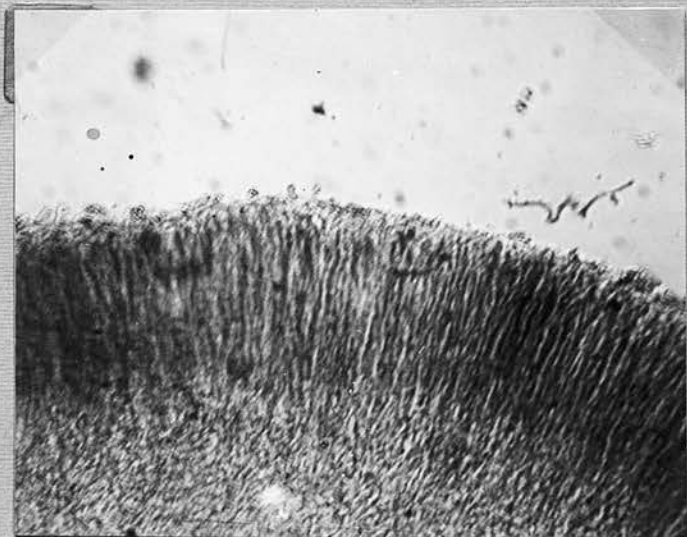
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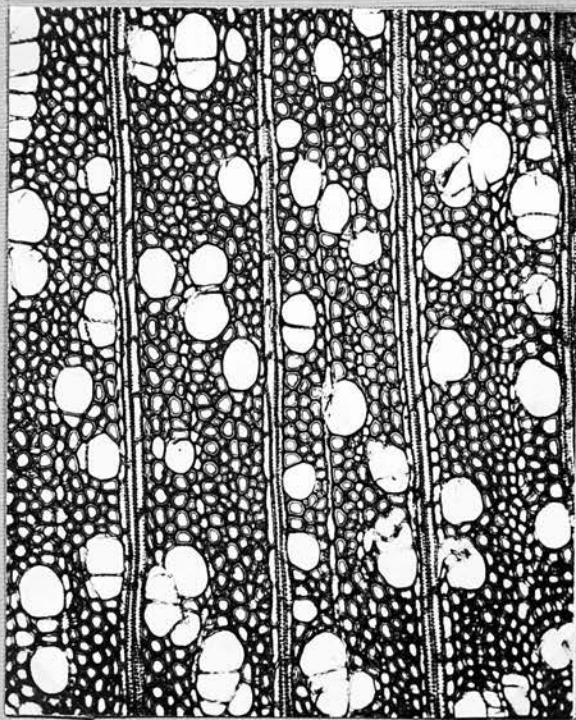
PLATE III.

Fig. 8. Part of a transverse section of normal elder wood. (x 100).

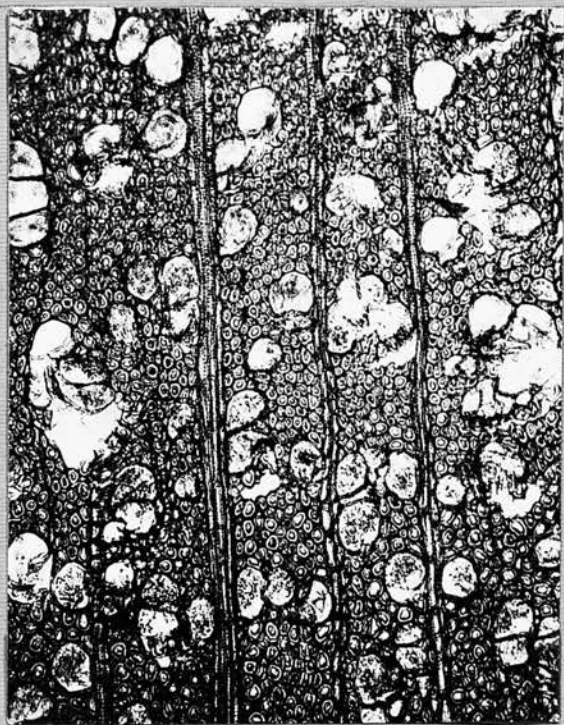
Fig. 9. Part of a transverse section of a partly decayed elder wood; masses of hyphae are visible within the vessels. (x 100).

Fig. 10. A magnified view of a cross section of partly decayed elder wood showing abundant hyphae within the wood-elements; (x 450).

Fig. 11. A general view of partly decayed elder wood in cross section showing distribution of the hyphae and spiral shrinkage cracks within the fibres. (x 100).



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PLATE IV.

Fig. 12. A general view of the wood in transverse section in an advanced stage of decay showing masses of hyphae in the vessel, the bore-holes and spiral shrinkage cracks in the fibres. (x 100).

Fig. 13. A vessel with hyphae penetrating through the bordered pits. (x 380).

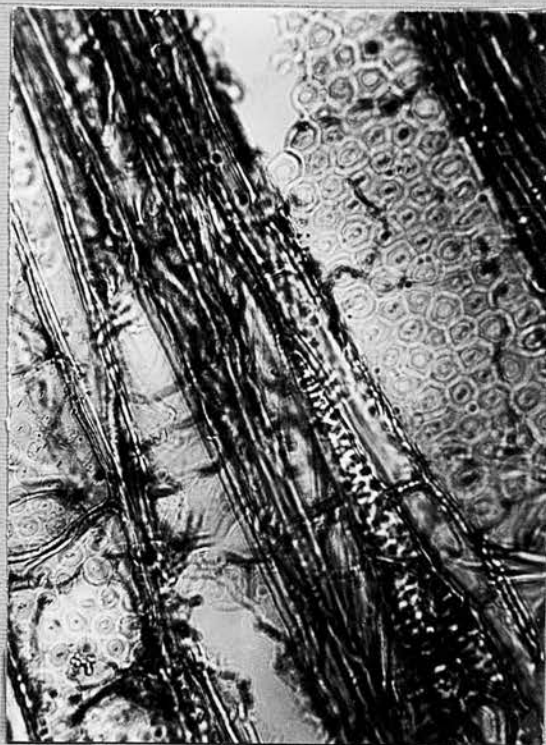
Fig. 14. Wider hyphae with clamp-connections passing through the pits. (x 900).

Fig. 15. A hypha penetrating the bordered pits and also the border part of a pit. (x 900)

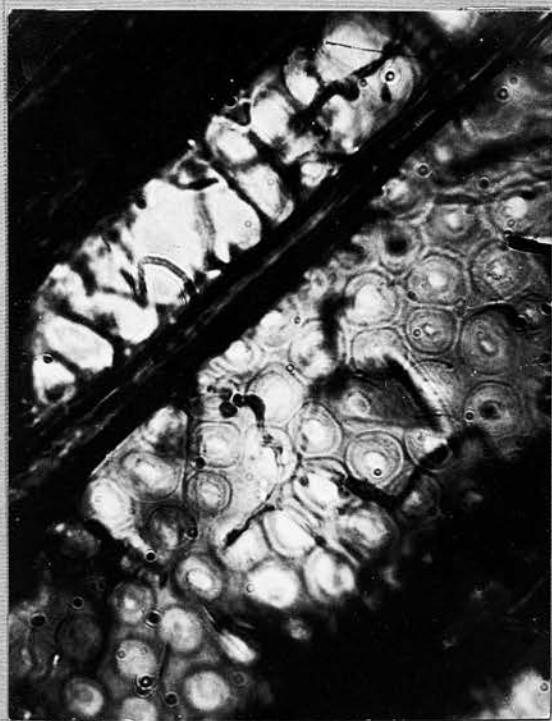




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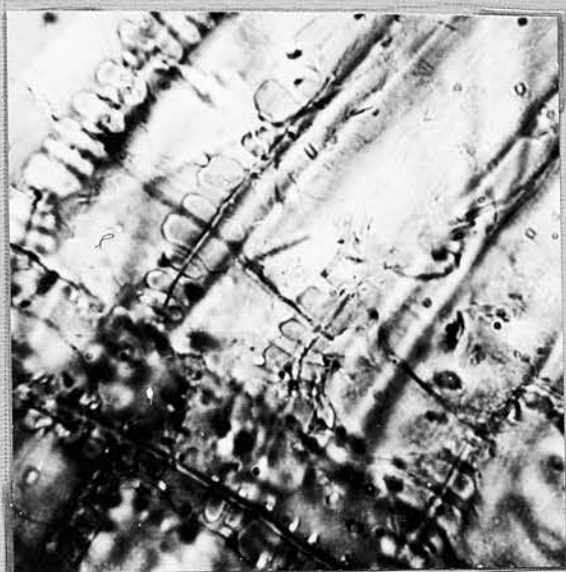


PLATE V.

Fig. 16. A hypha passing through the pits of the medullary ray cells. (x 900).

Fig. 17.-19. Completed penetration of the cell-walls by the hyphae (figs. 17, 18) after their emergence from the bore-holes. The apex of a larger hypha has become attenuated into the relatively fine point of considerably smaller diameter; it has reached the middle-lamella and is visible within the layer bore-hole (fig. 19). (x 900).

PLATE V



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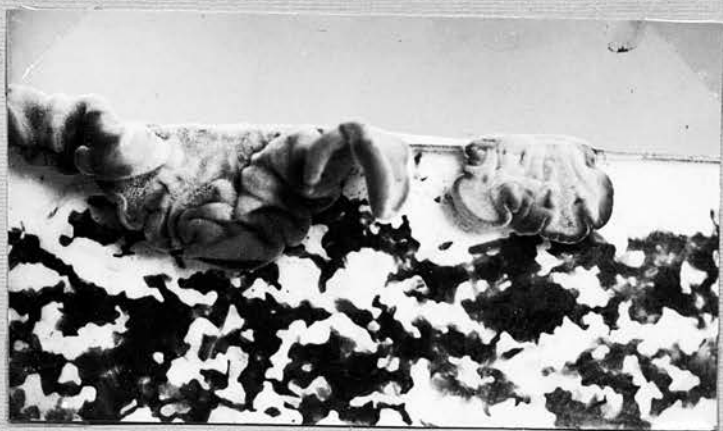
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PLATE VI.

Figs. 20 & 22. Fructifications of Auricularia  
auricula-Judae formed at the edge of the  
Petri-dishes in artificial culture.

(Figs. 20-21, x 4).

Fig. 23. Irregular fructifications formed  
at the rim of the test-tube within the  
flask.



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PLATE VII.

Fig. 24. 21-days-old culture of Auricularia auricula-Judae on malt agar at 23°C in darkness.

Fig. 25. Cultures of Auricularia auricula-Judae, 21-days-old, on potato-dextrose agar and malt agar in darkness and at 18°C (a, c) and (b, d) respectively.

Fig. 26. Wood-block cultures of Auricularia auricula-Judae, 30-days-old, for testing decay resistance in the laboratory.

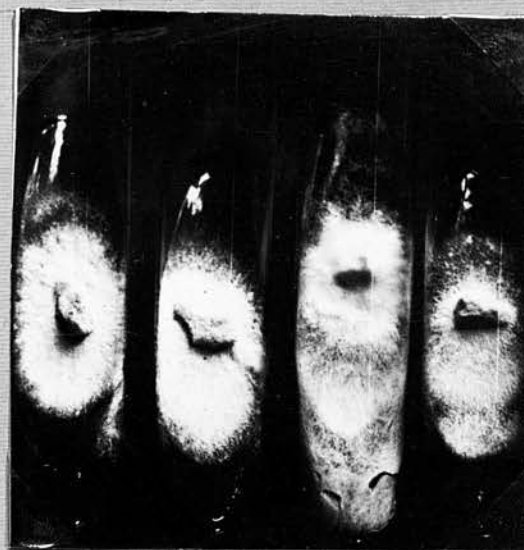
Fig. 27. Auricularia auricula-Judae showing positive oxidase reaction after 24 hours on malt agar containing 0.5 per cent gallic acid.

Fig. 28. Wood-blocks of elder showing decay after 8 months' exposure to Auricularia auricula-Judae

Fig. 29. Submerged mycelium of Auricularia auricula-Judae. (x 200).



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*a*

*b*

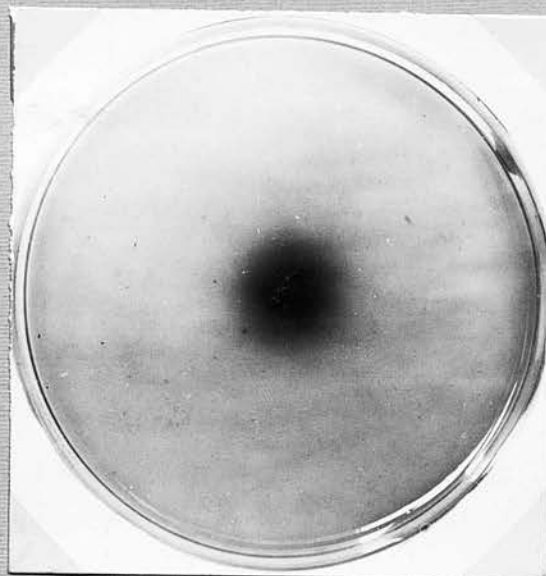
*c*

*d*

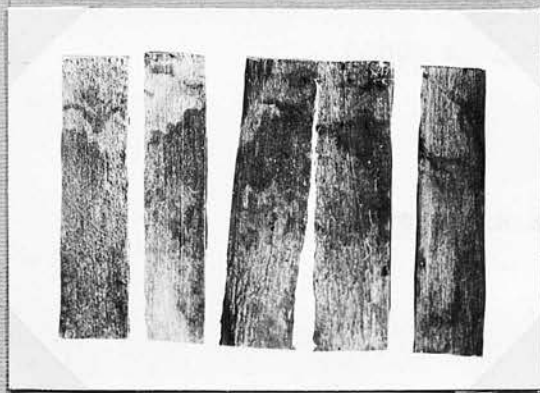
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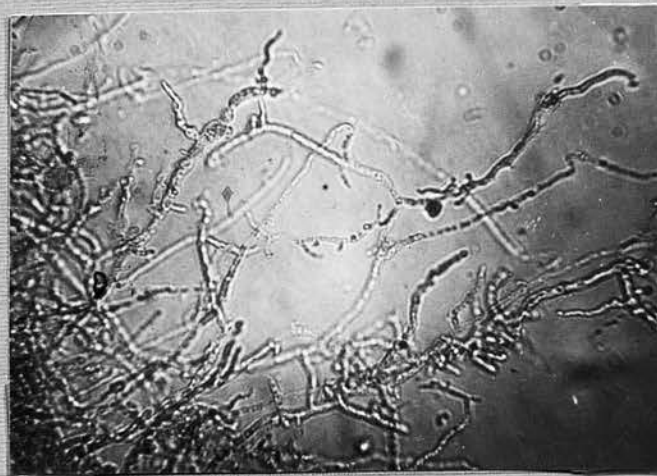
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PLATE VIII.

Figs. 30 & 31. Pairings of monosporeous cultures of Auricularia auricula-Judae showing: (a) space of aversion (fig 31), (b) slight aversion but the gap was filled with dicaryotic mycelium (fig. 30, b, c) and (c) even intermingling of the mycelia (fig. 30,a).

Fig. 32. Agar-film technique as used in this investigation. The two colonies on the agar film are two compatible haploids which are in the process of fusion.

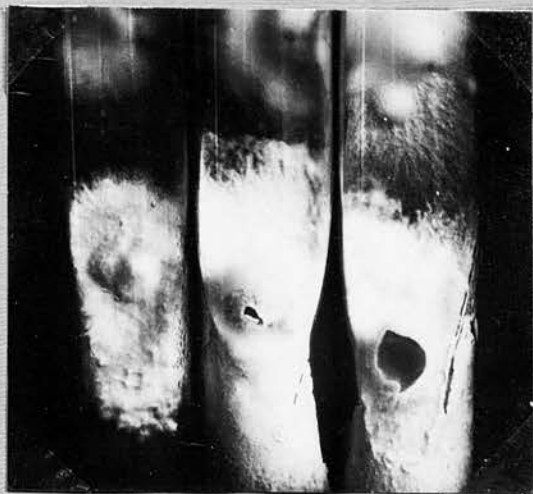
Fig. 33. Spores of Auricularia auricula-Judae germinating directly into mycelia on malt agar medium. (x 200).

Figs. 34 & 35. Germinating spores of Auricularia auricula-Judae producing conidia. (x 800).

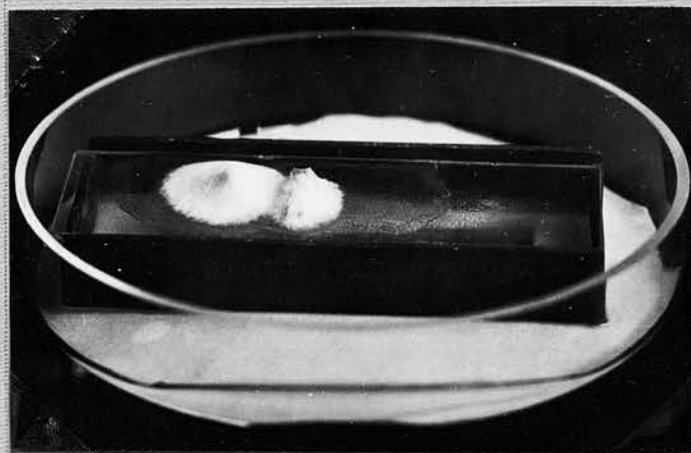




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PLATE IX.

Fig. 36. Inoculated twigs of Sambucus racemosa  
showing infections after 4 months.

Fig. 37. Inoculated twigs of Sambucus nigra  
showing the infection (b) and the  
control (a) after 4 months.



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III.      AN OAK (QUERCUS ROBUR LINN.)  
CANKER CAUSED BY STEREUM RUGOSUM  
(PERS.) FR.

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## INTRODUCTION.

Stereum rugosum (Pers.) Fr. has been known to cause a white pipe rot of oak (Quercus robur Linn.) and is regarded as a fungus of minor importance on standing trees in Great Britain (Cartwright and Findlay, 1936, 1946). In recent years attention of the writer has been directed to a destructive canker of oak in a private plantation (Balfour Estate) at Dawyck, south-west of Edinburgh, Scotland. After close examination of the diseased trees fructifications of a fungus were found on or near the cankered areas and the fungus was identified as Stereum rugosum (Pers.) Fr. As a result of further investigation it has been found that Stereum rugosum is responsible for such cankers on the trunk of oak. The affected trees were about 50 to 60 years old and growing in a mixed stand with larch, sycamore and beech. For the most part the oak trees appeared to be in good condition, but about a dozen of them showed cankers on their trunks. The infected trees looked unhealthy, their tops were poor and they were in the process of dying. The situation was rather damp and heavily shaded; the soil dampness and relatively high humidity of the air were suitable for such a fungal infection.

Although the causal organism has been known to occur in Great Britain for about 100 years or more, its activity as a parasite to form cankers on oak is

still unknown in this country. A somewhat similar canker caused by a species of Stereum was described by Potter (1901) from north of England and he named the fungus as Stereum quercinum. Such canker-formation is, however, known to occur on the continent of Europe. Eddelbuttel (1911) described canker-like wounds with fructifications of Stereum rugosum on a young oak and on an apple tree; these cankers resembled those on larch in outward appearance. It was Liese (1930), who first expressed the doubt that Potter's fungus might be Stereum rugosum. He described a canker of the oak with numerous fructifications of the fungus near the base of the trunk in a private wood near Berlin. A similar view was also held by Bergenthal (1933-34) who observed oak cankers at various places in Germany and found that in all cases Stereum rugosum was found to be the cause of the disease. Occurrence of similar cankers had also been reported from Denmark by Ferdinandsen and Jorgensen (1938-39).

The major purpose of this study is to describe and illustrate the canker disease and its causal organism. It has also been considered essential to determine, by means of artificial inoculation experiments in the field, the parasitic activity of the fungus and the amount of damage it causes to samples of oak wood under controlled conditions. The results obtained so far are given in the following pages.

II. SYMPTOMS.

The symptoms were generally conspicuous. The cankers on the living trees were somewhat oval but most often elongated with depressed centres and swollen margins and were associated with the decay. (Pl. I, fig. 2 ). There was considerable distortion of the trunk which rendered them conspicuous, especially when more than one canker occurred on the same trunk. These cankers, measuring 16-34x12-14 inches, were found at various heights on the stem from 1-6 feet or more from the base upwards. In some cases two cankers were found, one above the other, on opposite sides of the stem. (Pl. I, figs. 1, 4) Near the cankered area the trunk usually bulged out and there was localised flattening of the stem on one side. (Pl. I, fig. 2 ). In case of a comparatively young canker the remnant of the stub of a dead branch could be seen at the centre. (Pl. I, fig. 2 ). Numerous fructifications of Stereum rugosum developed either near the margin or on the central portion of the cankered areas, usually around the stub of the dead branch (Pl. I fig. 2 ). In an advanced stage of decay a cavity was formed being surrounded by a prominent callus ridge; its edge often bulged out owing to lateral extension. Eventually, a gaping wound surrounded by a raised callus-tissue was formed and never healed up. (Pl. I, figs. 1, 3, 4). In extreme cases of decay, both sap-wood and heart-

wood became discoloured, light, soft, somewhat spongy, and ultimately becoming brittle crumbled readily into flakes or powder on drying. Sometimes the sap-wood separated along the annual rings and sheets of whitish mycelium was found in between these cracks and between the bark and the sap-wood. Sections from such extreme decay showed that the summer-wood to be mostly a network of middle-lamellae and it was in this portion of the annual ring that the wood separated. A cross section through a canker (Pl. II, fig. 5 ) showed that the decay extended into the heartwood which was completely exposed, the sap-wood/<sup>outside the</sup>wound being completely decayed. In other cases, the sap-wood was found in a badly decayed condition. The progress of decay in the heartwood was rather comparatively slow. A narrow dark-brown invasion zone of the rotted heartwood was always visible towards the exposed surface of the canker. Tissues outside this zone appeared perfectly sound. Lateral extension at the edge of the canker to heal the wound was clearly visible. It was observed that after the formation of the canker no complete annual ring was formed. The wood in the upper and the lower regions of the cankered stem, however, appeared normal.



### III. MATERIALS FOR STUDY.

The materials were collected in January and July, 1950, in the form of freshly cut cankers from the infected oak trees. As the situation was somewhat shady, cool and damp, the fructifications of Stereum rugosum were found growing favourably on the surface of the cankers on the decaying trunks of living trees. The cankers were macroscopically examined in the field and their characteristics were noted as far as possible. For further study, portions of the cankered trunk were brought into the laboratory and studied in a fresh condition.

### IV. THE SPOROPHORE.

#### Description.

#### STEREUM RUGOSUM (Pers.) Fr.

Fructifications. - Usually resupinate and stretched out flat on the substratum (Pl. I, fig. 2 ) sometimes effused - reflexed with a narrow, free, upturned marginal pileus (Pl. II, fig. 6 ), diameter of the resupinate portion 1 to 6 c.m., reflexed portion about 2 to 10 m.m. deep and .5 to 2m.m. thick; often laterally confluent and the reflexed portion imbricate; leathery to corky, becoming hard on drying; margin thick,

entire, often obtuse, pale coloured.

Upper surface. - At first silky and Pinkish buff in colour; becoming glabrous and concentrically zoned with age; radially furrowed; Pinkish buff towards the margin but Drab gray to Vinaceous drab on weathering.

Context. - thin; coriaceous to rigid; whitish to yellowish.

Hymenial surface. - Pruinose, dull; bleeding red when fresh if cut or bruised; sometimes uneven; pale Pinkish buff to Pinkish buff, often becoming greyish with age.

Basidia. - Elongated, clavate; dimension about  $38-50 \times 5-6 (7.5) \mu$ .

Spores. - Hyaline; smooth, oblong and somewhat flat at one side, often slightly curved; dimension about  $8-12 \times 3-4.5 \mu$ .

Cystidia. - None.

Lactiferous cells. - Numerous, curving up into the hymenium from the underlying tissue; slightly flexuous; reddish-brown in colour; about  $3-6 \mu$  in diameter.

Tissue differentiation. - In structure the fructifications are about  $600-1600 (2000) \mu$  thick and the following regions can be

differentiated: (a) a hymenial layer, 2 to many zoned, about 100-700 (1300)  $\mu$  thick (Pl. II, fig. 7). (b) an intermediate layer, about 300-500  $\mu$  thick, and consisting of thick-walled, hyaline, closely septate, parallel hyphae, about 2.5 - 3  $\mu$  wide, mostly empty; (c) a golden-yellow zone, about 80-100  $\mu$  thick lying between the intermediate layer and the outermost hairy covering, composed of longitudinally arranged hyphae; and (d) the hairy covering, about 100-400  $\mu$  thick, consisting of hyaline or pale coloured, straight or slightly flexuous, septate hyphae, about 2.5 - 4  $\mu$  wide, often matted.

The above description has been based on materials growing on oak canker at Dawyck. The colours mentioned in the description are according to Ridgeway (1912). In this country, the fructifications of Stereum rugosum are commonly found on stumps, trunks and fallen branches of broad-leaved trees. The bleeding hymenium and the presence of dark coloured conducting organs places this fungus near Stereum sanguinolentum and Stereum gausapatum. It can be readily distinguished from them by its characteristic multizonate hymenium and more woody texture.

V. DECAY OF OAK.(a) Microscopic details of the rot.

In order to determine the extent of damage caused by the fungus within the host tissue, small pieces of sound and infected wood in various stages of decay were sectioned both free hand and with the microtome. Transverse, radial and tangential sections of the wood, about 15 to 20  $\mu$  thick, were cut only after softening the materials by immersing the blocks in a vessel of gently boiling water until they became water-logged and sank. The sections, thus obtained, were preserved in 50% alcohol for future use. As it was very difficult to observe hyphae in the sections of the heartwood in an incipient stage of decay, differential staining was made of the sections by safranin and picro-aniline-blue as described by Cartwright (1929) with satisfactory results.

In an incipient stage of decay caused by Stereum rugosum the mycelium is rather scanty but as the decay advances, it becomes distributed throughout all the elements of the heart-wood, being particularly plentiful in the vessels, medullary rays and wood parenchyma (Pl. V, figs. 22, 23). The hyphae, sometimes filling entire cavities of the fibres, wood-parenchyma and ray cells and forming compact masses, are rather fine, profusely branched,



usually hyaline, occasionally yellowish-brown, about 1-3  $\mu$  across and with infrequent clamp-connections. The hyphae within the fibres and tracheids are often seen to run in a more or less longitudinal direction. A gradual thinning out of the secondary walls of the fibres from the lumen towards the middle lamellae is evident but less commonly in the tracheids (Pl. V, figs. 24, 25). The vessels, wood-parenchyma are least affected, although the tyloses are often broken down. (Pl. V, figs. 24, 25). The wood parenchyma cells scattered among the fibres and tracheids show little evidence of decay, excepting the presence of numerous bore-holes through which the hyphae pass. The hyphae at first ramify through the pits but later directly through the cell-walls forming numerous bore-holes. These bore-holes, which are abundant in an advanced stage, are at first circular but later more or less elongated and about 1-4  $\mu$  across. Dark coloured, brown to blackish gummy materials are present in the parenchyma and ray cells. In case of extreme decay, the wood presents the appearance of a network of middle lamellae but the vessels, rays and wood parenchyma are still recognisable. The elements break apart easily and can be teased out for examination. On being treated with phloroglucin and hydrochloric acid they turn pink which indicates that some amount of lignin is still present.

(b) Microchemical Studies.

It has been mentioned in a previous paper (Banerjee, 1951) that although microchemical tests may lead to erroneous conclusion, they are undoubtedly useful as indicators of the presence of lignin and cellulose and as such they are still commonly used in such work.

Results of two microchemical tests for cellulose and lignin in the normal and partly decayed heartwood will be recorded here. This has been done by staining the sections with chlor-zinc-iodine for cellulose and phloroglucin and hydrochloric acid for lignin. Other microchemical reagents have also been employed and the results of these agree closely with the changes indicated by the use of the above-mentioned reagents. The sap-wood near the outer edge of the canker appeared to be sound and was completely occluded by the callus ridge and as such partially decayed sapwood could not be obtained for such studies.

Table 1/

TABLE I. Results of staining for cellulose and lignin in normal and partially decayed heartwood of oak (*Q. robur*) with Chlor-zinc-iodine and Phloroglucin-HCl respectively.

Cellulose (Chlor-zinc-iodine)		
Elements	Normal heart-wood	Decayed heartwood
Vessels.....	Tyloses faint blue to sometimes colourless.	Tyloses broken down at places; remnants faint blue to colourless.
Fibres.....	Secondary walls mostly faint blue, often colourless.	Secondary walls light blue to colourless, particularly near the lumen; sometimes entirely light blue and often blue at places.
Tracheids...	Secondary walls in some cases light blue.	Secondary walls in some cases faint blue to almost colourless.
Wood parenchyma..	End-walls at times blue.	Secondary walls in some cases light to faint blue; end walls often colourless.
Ray cells....	Some secondary walls light blue.	Secondary walls often light blue or lavender.

Lignin/

Table 1 continued.....

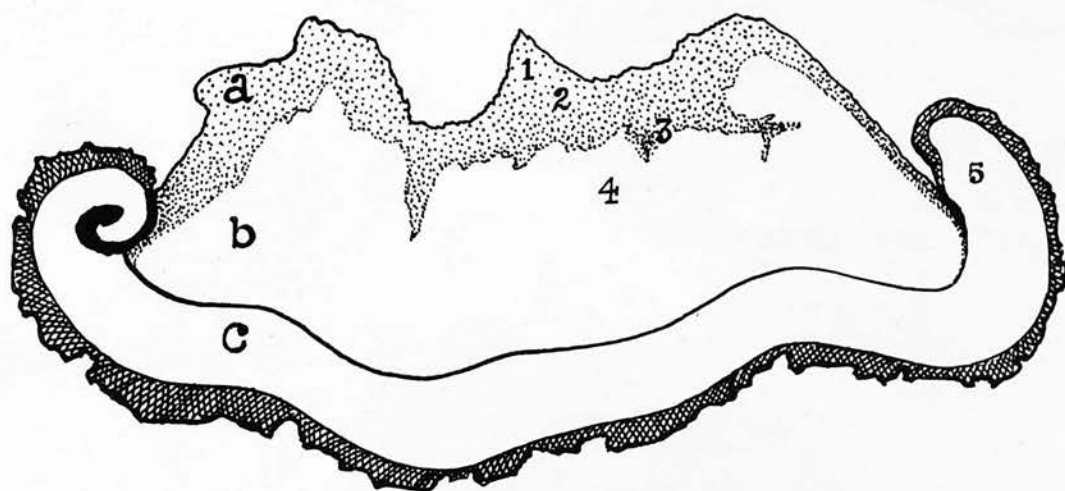
Lignin (Phloroglucin-HCl)		
Elements.	Normal wood.	Decayed heartwood
Vessels	Tyloses red to colourless; primary and secondary walls deep red.	Tyloses pale pink to colourless; primary and secondary walls red to light red.
Fibres	Primary walls red; secondary walls light red to pink, often colourless.	Primary walls light red to pink; secondary walls mostly pink to colourless.
Tracheids	Primary walls red; secondary walls red near middle lamella, pink to colourless near the lumen.	Primary walls red to light red; secondary walls mostly pink to colourless.
Wood parenchyma	Primary walls red; secondary walls light red.	Primary walls light red; secondary walls pale pink.
Ray cells.	Mostly red.	Primary walls red to light red; secondary walls mostly pink, sometimes colourless.



From table 1 it will be evident that the fungus can gradually digest both cellulose and lignin during the process of decay, the heavily lignified parts being partially affected. Fahraeus et al (1945), while studying the decomposition of wood by some white rot fungi, have shown that Stereum rugosum is more specialised on cellulose, and attacks especially in the later stages, cellulose in preference to lignin. In oak the tyloses are lignified structures and give positive lignin reaction. Sometimes the faint blue colour with chlor-zinc-iodine may possibly be explained as due to the presence of a thin secondary wall of cellulosic material which responds to the test. Although broken down at places, they are present in an advanced stage of decay being filled with hyphae and perforations in their walls but these usually fall apart during the process of sectioning.

VI. CULTURE STUDIES.

Stereum rugosum was grown in culture and their characteristics studied on two different media, viz., potato-dextrose agar and 2.5% malt agar. The pH value of each medium was adjusted and after sterilization was found to be 5.2. The initial cultures were made by the use of spores from fresh sporophores found on the surface of oak cankers and from portions of the decayed heartwood. The methods for making cultures had already been described (Banerjee 1951). All cultures were grown in test tubes, 6" x  $\frac{3}{4}$ " in size and kept in darkness in different incubators at 18°C and 23°C respectively. For determining the rate of growth the mycelium was however, grown in Petri-dishes (90 m.m. in diameter). In most cases the mycelium grew out in the course of a week and from these sub-cultures were made in malt agar tubes which served as stock cultures. Tissue cultures were obtained from the areas designated by the numbers 1, 2 and 3, while from areas 4 and 5 no cultures could be secured (text-fig. 1). Microscopic examinations of the sections taken from these regions showed that hyphae were not present in the areas 4 and 5. It is, therefore, obvious that the hyphae could not penetrate far beyond the invaded zone. After comparison it was found that



Text-fig. I. Diagrammatic representation of a cross-section through an Oak Canker showing different regions; a, the zone of decayed heartwood; b, sound heartwood; c, sapwood. The numbers indicate regions from which cultures of the fungus were attempted.

the cultures obtained from the infected wood were identical with the polysporous ones in cultural and microscopic details.

The colour exhibited by the mycelium during growth has been expressed according to Ridgeway (1912) and the terms used to describe the texture of the mat are those of Long and Harsch (1918).

(a) Oxidase tests.

Bavendamm (1928) described a test by which dark rings appear around the inocula due to the presence of oxidases characteristic of the white rot fungi and these can be detected by growing the fungi on malt agar medium containing 0.5 per cent gallic or tannic acid. This test has been performed with Stereum rugosum and it has been found that it, being a white rot fungus, can produce such rings and therefore capable of decomposing lignin with other substances. The intensity of reaction was however, at first mild on gallic acid medium but later it became intense. The diameters of the rings after 48 and 72 hours were 12 and 15 m.m. respectively, <sup>(Pl. III, figs. 11, 12).</sup> The fungus can also give positive reaction to the test recommended by Preston and McLennan (1948) and partially bleach the colour of gentian violet present in the medium but about 6 weeks' incubation is necessary before definite results can be obtained.



(b) Cultural characteristics.Stereum rugosum (Pers.) Fr.

- (1) Habit of growth.- (Pl. III, figs. 13-16). On potato-dextrose agar at 18°C the growth started with much rapidity and within two days after inoculation thin cottony mycelium appeared over the inoculum and with an appressed and sodden growth, about 3-4 m.m. across, over the surface of the slant. Condensation of the mat started early and in about 5 to 6 days this inoculum turned felty but the mat proper became differentiated into a broad cotton-woolly zone, about 5-6 m.m. wide and an appressed, colourless zone of advance. As the growth advanced, there was a felty ball over the transplant and the whole surface of the slant became covered in about a week with a smooth, soft, homogeneous and felty mat. After a month the surface of the mat became uneven due to the formation of small, raised, felty areas. The medium was discoloured and became pale yellow in cultures, 10-days-old. No marked difference in cultural characteristics was, however, noticed in cultures grown at 23°C, excepting in that there was early condensation of the mycelium to form a smooth, even and felty mat. On malt agar, on the other hand, at 18°C the growth was poorer than that on potato-dextrose agar. There was not much aerial mycelium

and its condensation was somewhat delayed. It was for the most part thin and cotton-woolly with an indistinct and narrow advancing zone and the inoculum was somewhat sub-felty. In about 10 days the lower half of the slant tended to become felty which later became entirely so towards the end of third week. The colour of the medium changed somewhat pinkish in cultures, 10-days-old. At 23°C, the growth was more or less the same as that at 18°C but aerial mycelium was formed and part of it became cottony within 5 days after inoculation.

- (ii) Colour - On potato-dextrose agar, colouration appeared first in about 5 days after inoculation over the transplant as a patch of Light buff or Salmon buff. The central portion of the mat turned Pinkish buff in cultures, 10-days-old, other portions remaining white till the end of fourth week when Light buff colour appeared on the lower half of the mat around the inoculum. At 23°C the pigmentation of the mat was more or less the same but patches of Light buff colour appeared over the white mat in cultures 35-days-old. On malt agar, on the other hand, colouration at both temperatures first appeared after a fortnight, only over the transplant as a patch of Warm buff. Later, Light buff and pale Antimony yellow appeared throughout the whole mat in both cases but it seemed

to be more pronounced in cultures kept at 18°C.

- (iii) Rate of growth. - The rate of growth, both on potato-dextrose agar and malt agar kept under identical conditions, was more or less the same and the daily increment in diameter of Petri-dish cultures was determined. In all cases average measurements were taken and found to vary between 9.5 and 10.5 m.m.

- (iv) Hyphal characters. -

Aerial mycelium: two types of hyphae can be recognised, viz., (a) wider thin-walled, hyaline hyphae, about 3-4  $\mu$  across, comparatively few, more or less straight, distantly branched and septate, with occasional simple clamp-connections, H - connections frequent; and (b) thin-walled, narrow hyphae, about 1-2.5  $\mu$  wide, numerous, much branched, branches originating usually at right angles to the main hypha, distantly septate, straight or flexuous, often spirally coiled, clamp-connections rare (Pl. IV, fig. 17).

Submerged mycelium: the same two types of hyphae can be recognised but with slight differences as follows:- (a) wider hyaline hyphae more frequent, about 4-5(6)  $\mu$  wide, much branched, septate closely or infrequently, straight or flexuous, with coarsely granular contents, often sparse, clamp-connections

rare; and (b) finer, much branched, flexuous hyphae, about  $1.5-3\ \mu$  wide, with coarse granular contents, hyaline or with a yellowish tint, clamp-connections rare, originating from the wider hyphae and often clustered (Pl. IV, fig. 18).

(c) Production of fruit-bodies in culture.

Cultures of Stereum rugosum grown in test tubes did not form fructifications on various media such as malt agar, potato-dextrose agar and oatmeal agar. However, while making decay resistance tests with oak samples in Erlenmeyer flasks, small resupinate fructifications appeared on the surface of the malt agar slant near the bare glass surface in cultures about 4-months-old. (Pl. II, fig. 8). The medium for developing sporophores recommended by Badcock (1941) was then tried. Sawdusts of beech and spruce, mixed separately with Badcock's 'accelerator' in varying proportions (5-40% by weight) after being soaked in water, were used in Petri-dishes, test-tubes (20 x 4 c.m.) and Badcock's apparatus (Badcock, 1943) and the various devices adopted for obtaining the sporophores had already been described (Banerjee, 1951). After sterilization the media were inoculated by the fungus and the cultures were kept in darkness at room-temperature ( $18^{\circ}\text{C}-21^{\circ}\text{C}$ )



inside a closed chamber in which a moderately high humidity was maintained. Subsequently, when the mycelium entirely covered the surface of the medium or permeated through the medium within the tubes, all cultures were removed and placed in strong diffused light on a table about 8 feet from a large window of the laboratory. The fungus made exceptionally rapid and luxuriant growth on the sawdust medium, particularly on those containing 30-40 per cent by weight of the 'accelerator'.

It was only in Petri-dish cultures with 30-40% 'accelerator' that the mycelium began to form small, fertile, resupinate fructifications, sometimes with a narrow, marginal, hairy pileus near the rim of the Petri-dish in about 3-4 months after inoculation. (Pl. II, fig. 9 ). The upper surface was somewhat greyish but the hymenial surface was light buff in colour. In test-tubes the fruit-bodies were not formed although there was much condensation of the superficial mycelium near the opened mouth of the tube. In Badcock's apparatus, on the other hand, irregular resupinate growth was noticed outside the rim of the test-tubes within the flask, in about 5-6 months. (Pl. II, fig. 10 ) These did not develop further, possibly due to limited space and too high a humidity within the flask.

VII. DECAY RESISTANCE TESTS IN THE  
LABORATORY.

In order to determine the natural resistance to decay of oak samples caused by Stereum rugosum under controlled conditions, wood-block cultures of the fungus were made in 500 c.c. Erlenmeyer flasks, each containing 250 c.c. of 2.5% malt agar which was slanted after sterilization. These flasks were then inoculated with bits of actively growing mycelium and were incubated at 23°C in darkness. Within a week the mycelium nearly covered the surface of each slant and was ready to receive the test-pieces.

The blocks for durability tests were uniformly cut from sound wood,  $\frac{1}{2} \times \frac{1}{2}$  inch square and 2 inches long, with the long axis parallel to the grain of the wood. The heartwood and sapwood were tested separately and close attention was paid in getting representative materials. These were then planed and serially numbered on all faces with a soft lead pencil. In order to determine the actual loss in weight due to decay these were dried to a constant weight in an oven, maintaining a temperature of 60°C. Before sterilization, these blocks were put in each Roux tube, having distilled water at the bottom and sterilized in an autoclave at 15 lbs. pressure for 10 minutes. The idea of

sterilizing the wood blocks in this way was to keep the atmosphere around them more or less saturated during the process so that little loss of water could take place from the wood blocks and consequently lesser time was required for sterilization as pointed out by Chidester (1937,1939). The test-pieces were then taken out individually and exposed to fungal action by placing them on cultures within the flasks. These were then kept at a constant temperature of 23°C in darkness. The period of exposure to fungal attack was 4 months. After the completion of the test, the blocks were taken out of the flasks, the superficial mycelium was carefully removed without damaging the wood blocks, weighed and again dried to a constant weight as before. The resulting dry weights subtracted from the original and the percentage loss, based on dry weight of the sound material, were calculated. The average moisture content of another set of sapwood and heartwood blocks before and after they were exposed to fungal action were determined in the same way. This was necessary to ensure that the moisture content of the wood-blocks was much above the fibre-saturation-point which is essential for the satisfactory growth of the wood-rotting fungus. The <sup>average</sup> moisture contents of the heartwood and the sapwood, after sterilization

were found to be 55.5% and 90.2% respectively. As the experiment was conducted for a limited period, no attempt was made to control the moisture content after they were exposed to fungal action in order to establish its relation with the progress of decay.

The growth of the mycelium on wood-blocks was incidentally noted. Within a month after inoculation all the faces of the sapwood blocks were completely covered with vigorously growing dense, uniform, cotton-woolly mycelium which later became felty. (Pl. IV, fig. 19 ). The mycelium was mostly white but light buff colour appeared in patches with the age of the culture. Considerable growth of the mycelium on to the bare glass surface due to vigorous growth at the edge of the slant was in evidence. On heartwood blocks, on the other hand, even after a month the growth was rather poor, thin, cotton-woolly and patchy, becoming somewhat felty at places (Pl. IV, fig. 20 ). Pigmentation of the white mycelium on the wood-blocks was, however, not in evidence.

The results of the experiment are given in tables 2 and 3.



Table 2. Decay resistance of sapwood of Oak after 4 months' test exposed to Stereum rugosum.

No. of Blocks.	Initial oven-dry weight (Grms) before test.	Final oven-dry weight (Grms) after test.	Loss	Loss %
1	4.329	3.926	.403	9.3
2	4.265	3.847	.418	9.8
3	4.322	3.770	.552	12.8
4	4.024	3.610	.414	10.3
5	4.485	3.999	.486	10.8
6	4.559	3.979	.580	12.7
7	4.290	3.780	.510	11.9
8	4.315	3.853	.462	10.7
9	4.593	4.172	.421	9.2
10	4.719	4.208	.511	10.8
11	4.284	3.791	.493	11.5
12	4.387	3.941	.446	10.2

Average loss in dry weight ( $\frac{\%}{2}$ )..... 10.8

(Range: 9.2 to 12.8)

Table 3. Decay resistance of heartwood of oak after 4 months' test exposed to Stereum rugosum.

No. of blocks.	Initial oven-dry weight (Grms) before test.	Final oven-dry weight (Grms) after test.	Loss	Loss%
1	4.261	4.013	.248	5.8
2	4.392	4.124	.268	6.1
3	4.690	4.394	.296	6.3
4	4.467	4.194	.273	6.2
5	4.120	3.859	.261	6.3
6	4.240	3.977	.263	6.2
7	4.151	3.858	.293	7.1
8	4.430	4.159	.271	6.1
9	4.515	4.253	.262	5.8
10	4.222	3.941	.281	6.7
11	4.521	4.253	.268	5.9
12	4.526	4.253	.273	6.0

Average loss in dry weight (%)..... 6.2

(Range: 5.8 to 7.1)

When the respective values of the losses in weight due to Stereum rugosum are studied in a comparative way the distinction is obvious. It is evident that the loss of weight in the heartwood is less than that in the sapwood. The loss suffered by the sapwood is quite significant as the test-pieces have lost a moderately high percentage of their original weights during the test and it can be safely concluded that the sapwood has low resistance to decay. The loss in the heartwood, on the other hand, is much less and the wood appears to be moderately resistant to decay. Therefore, the sapwood and the heartwood of oak may be classed under Findlay's (1938) 'non-resistant' and 'moderately resistant' groups of timbers respectively according to their natural resistance to decay caused by Stereum rugosum; the former suffers average losses in weight from 10-30% and the latter up to about 10% during four months' test. It is to be pointed out here that the average moisture contents of the sapwood and heartwood blocks after completion of the test were 131.3% and 83% respectively and this rise in water contents was obviously due to absorption of water from the agar medium on which they were placed.

VIII. FIELD INOCULATIONS.

Although Stereum rugosum was isolated from the decayed tissues of the cankered oak and grown successfully in the laboratory on heartwood, sapwood and agar media, it was considered desirable to ascertain whether the fungus could infect the living trees by artificial inoculation in the field. During summer of 1950, two inoculations were made on the trunk of healthy Quercus robur, about 20-year-old, located in a private plantation at Dawyck, south-west of Edinburgh, Scotland. The operation involved in inoculating the oak tree, consisted in transferring aseptically small bits of agar with actively growing mycelium from test tube cultures into a wound made by making a cross incision, about 2" x 2", through the bark so as to obtain a flap of the bark still attached to the trunk. Before making the incision the surface of the bark was wiped out with absolute alcohol and then with sterile distilled water in order to remove the surface contaminations as far as practicable. Equal number of controls were established on the same tree, placed on the trunk either above or below the inoculations. After putting the inoculum under the flap, the inoculations and the controls were protected by placing a moist sterile cotton wool above each incision and



covering the whole with thick brown paper tied with a string. These were, however, later removed and both the checks and the inoculations were left exposed.

After a lapse of four months the wound inoculations were found uniformly positive (Pl. VII, figs. 26, 27) while the controls remained flat and healed up. The surface of the bark around the infected regions was swollen, somewhat soft, slightly discoloured and the wound was hollow and gaping open at the centre. The callus tissue was not formed and this apparently showed that within a short time sufficient mycelium had been produced to parasitize the living bark and the underlying tissues.

In March, 1951, the tree was again examined and the inoculated portions were removed, <sup>(Pl. VII, fig. 28).</sup> A cavity about one inch wide, two inches long and

half an inch deep had been formed, The spread of the mycelium is limited by a narrow, brown, rotted zone in the sapwood. Mycelium has been found in all the elements of the wood.

IX. DISCUSSION.

In this study evidence has been presented to demonstrate that Stereum rugosum acts as a virulent canker producing parasite on the common oak in a damp, heavily shaded situation. This pathogen is widely distributed in Great Britain and in the continents of Europe and America, usually growing as a saprophyte on stumps, trunks and fallen branches of broad leaved trees. This organism, generally believed of minor importance on standing trees, under damp conditions has become an aggressive parasite and caused destructive cankers on the oak.

There is reason to believe that the fungus has invaded the bark of the trunks and become established through wounds due, possibly, to mechanical injuries and stubs of dead twigs. Close examination of the young cankers has revealed that these have invariably developed around stubs of dead branches which have been badly decayed by the activity of the invading pathogen and the cavities are filled with plugs of soft decayed materials. Furtifications of the fungus have been invariably found on the surface of the bark around the stub in young cankers. That the fungus is able to infect healthy, living trees through wounds in the bark has been positively confirmed by inoculation experiments in the field. As the result of these experiments it can, therefore, be concluded that Stereum rugosum is a wound parasite

and is able to attack living oak trees even under normal conditions. That the sapwood of oak is non-resistant to decay and readily attacked by Stereum rugosum has been proved by experiments on durability tests in the laboratory. In all cases the cankers have shown that it is the sapwood which is most affected and has been completely destroyed forming an open wound exposing the heartwood. The heartwood, on the other hand, is relatively resistant to decay and in the open wound the progress of decay has been found to be very slow, being confined only to a limited, narrow, dark coloured invading zone beyond which the heartwood appears perfectly sound.

It has already been mentioned that Potter (1901) described a similar type of canker-formation on oak from northern England and the causal organism was identified by him as a new species of Stereum which he named Stereum quercinum. Both Liese (1930) and Bergenthal (1933-34) questioned the identity of S. quercinum Potter and they suggested that Potter's fungus might be Stereum rugosum. They found similar cankers in different places in Germany and in all cases the causal organism was identified as S. rugosum. According to Rea (1922), Stereum quercinum Potter is synonymous with Stereum spadiceum Fr. and Stereum gausapatum Fr. Recent work by Davidson (1934) has shown that S. spadiceum



and S. gausapatum are probably synonymous and they are responsible for the heartrot in various species of oak in America. In Great Britain S. spadiceum is known to cause a serious pipe rot in oak, gaining entrance through a large branch stub and spreading up and down the trunk. If it is taken for granted that S. quercinum Potter is synonymous with S. spadiceum, then the fungus, responsible for the canker in question, is quite different from Potter's fungus and from S. spadiceum in having a light coloured multizonate hymenium and more woody and usually resupinate fructifications; they have one characteristic however, in common i.e. the presence of numerous lactiferous cells in the hymenium which bleeds when cut or bruised. The fungus in question has been identified as Stereum rugosum (Pers.) Fr. and these characteristic features distinguish it from all other allied species of Stereum. Since the publication of Potter's account on oak canker, S. quercinum has never been collected since and identified as such, while S. rugosum is a common native on oak and other broad leaved trees. On account of these facts and from the descriptions of the disease now given and that given by Potter, it may be suggested that the oak canker described by Potter was possibly caused by Stereum rugosum. This fungus which has been reported from Germany as causing pipe rot, has given rise to destructive cankers,

possibly due to damp conditions which favour their development.

At present no positive method of cure for severely cankered trees can be suggested as they are almost sure to die. In order to reduce the danger of spread to other oak trees, the cankered trees should be removed from the plantation; this will make openings in the crown cover and thereby alter the density of the plantation and humidity of the situation. The affected and dead branches should be removed from the trunk and any open wound should be covered with a paint in order to protect the surface from moisture which favours fungal spores to germinate on the surface causing infection. An alternative method is to apply a fungicide to the cut surface to kill any superficial spore. As both vigorous and non-vigorous trees are susceptible to the disease under unsuitable conditions, thinning would be advisable in order to admit more light and ventilation into the plantation.

X. SUMMARY.

1. A hitherto unreported destructive oak canker in Scotland and its causal organism, Stereum rugosum, have been studied and described from Dawyck in Peeblesshire. Under certain conditions the disease may become destructive.
2. The cankers have been found on trunks of trees about 50 to 60-years-old. The situation, on the whole, is damp and shady.
3. The cankers are mostly open with a depressed to hollow centre which is surrounded by a prominent callus ridge and are often laterally extended. Both sapwood and heartwood have been attacked and these gradually become discoloured, soft, spongy, ultimately brittle, and crumble readily into flakes or powder on drying.
4. The fructifications of Stereum rugosum have been found at the edge or near the centre on the surface of the dead bark, usually around the stub of a dead branch.
5. Microscopic characters of the rots have been fully described. It has been observed that within the rotted tissues the mycelium gradually becomes distributed throughout all the elements of the wood, being particularly plentiful in the vessels, medullary rays and wood-parenchyma. Numerous bore-holes are present in the walls which are in an advanced stage of decay.

6. Chemical changes in the wood during the process of decay have been tested with the usual micro-chemical stains; they show that the causal organism can digest both lignin and cellulose. Being a white rot, the fungus gives the positive oxidase test on tannic acid or gallic acid medium.
7. Stereum rugosum has been easily obtained in pure culture from areas of both advanced and incipient decay and also from spores; its characteristics have been studied on potato-dextrose agar and malt agar. It can be easily identified by its characteristic dense, white, cotton-woolly to felty mycelium, rapid growth and distinctive buff or pinkish buff colour of the mat with age.
8. Inoculations, made on the wounded bark on the trunk of a healthy oak tree employing mycelium as the inoculum, have proved positive and formed gaping wounds. The controls have remained flat and healed up satisfactorily. This indicates that Stereum rugosum is capable of infecting living trees through wounds in the bark and it can, therefore, be regarded as a wound parasite on normal vigorous trees.
9. The relative resistance to decay of the sapwood and the heartwood of oak by S. rugosum has been determined in the laboratory. It has been found that the fungus is able to attack the sapwood much



more readily than the heartwood. The average loss in weight caused in the sapwood during 4 months' test has been found to be greater than that in the heartwood.

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# PLATES



PLATE I.

Fig. 1. General appearance of a heavily cankered oak (Q. robur) in the plantation; two cankers are seen situated one above the other.

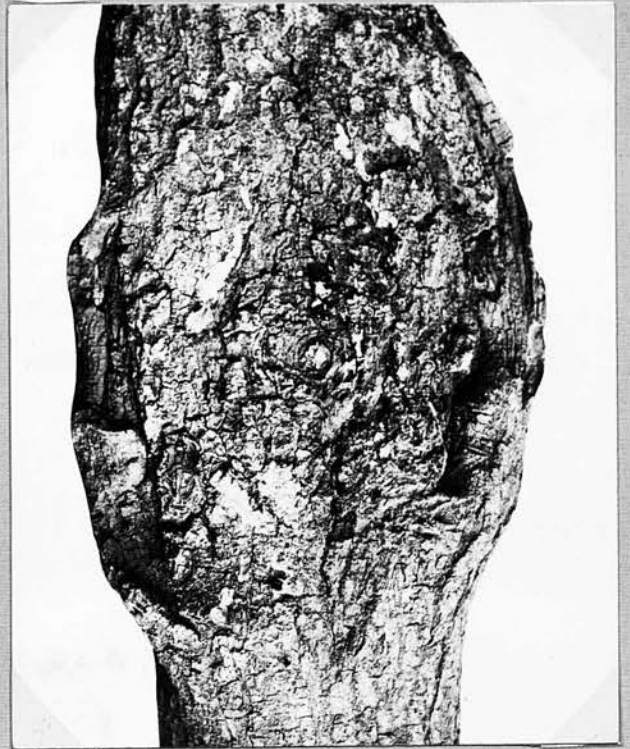
Fig. 2. A comparatively young canker showing characteristic swelling and flattening of one side of the trunk; resupinate fructifications of Stereum rugosum can be seen on the decaying bark around the rotten branch stub at the centre.

Figs. 3 & 4. Cankers showing advanced stages of decay; sapwood has been completely destroyed exposing the heartwood which is also partially decayed. The prominent and raised callus ridge surrounding the wound is evident.

PLATE I



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2



3



4

PLATE II.

Fig. 5. A cross section through a canker showing an advanced stage of decay; the exposed heartwood is partially decayed showing a narrow decayed zone of advance beyond which the heartwood and the sapwood appear perfectly sound. The callus ridge will be seen to occlude the sapwood near the edge from decay.

Fig. 6. Effuso-reflexed fructifications of Stereum rugosum found growing near the margin of the canker.

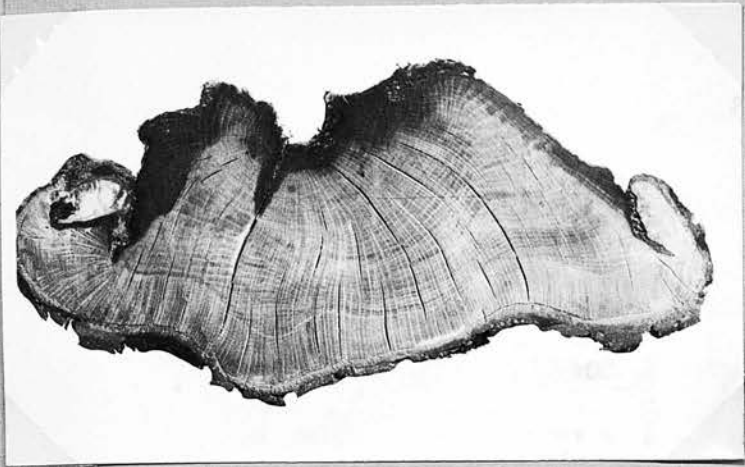
Fig. 7. A cross section through the fruit-body of S. rugosum showing the multizonate hymenium.(x 100).

Fig. 8. Wood-block cultures of S. rugosum, 60-days-old, for testing decay resistance in the laboratory; a resupinate fructification has developed on the agar surface.

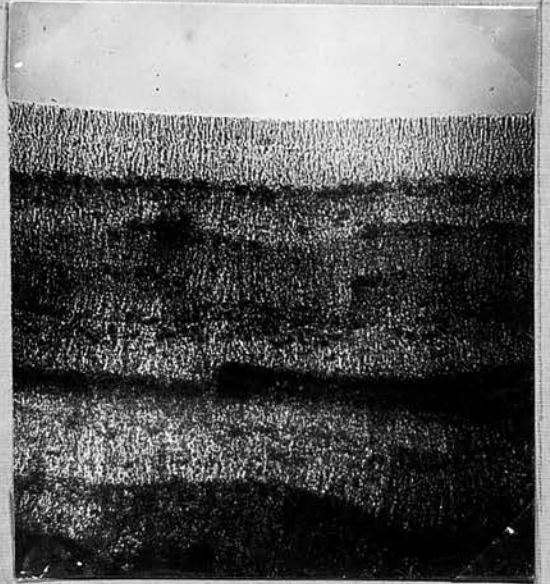
Fig. 9. Resupinate and effuso-reflexed fructifications of S. rugosum near the rim of the Petri-dish in artificial culture.

Fig. 10. Irregular fructification of S. rugosum formed at the edge of the test-tube within the flask in culture.

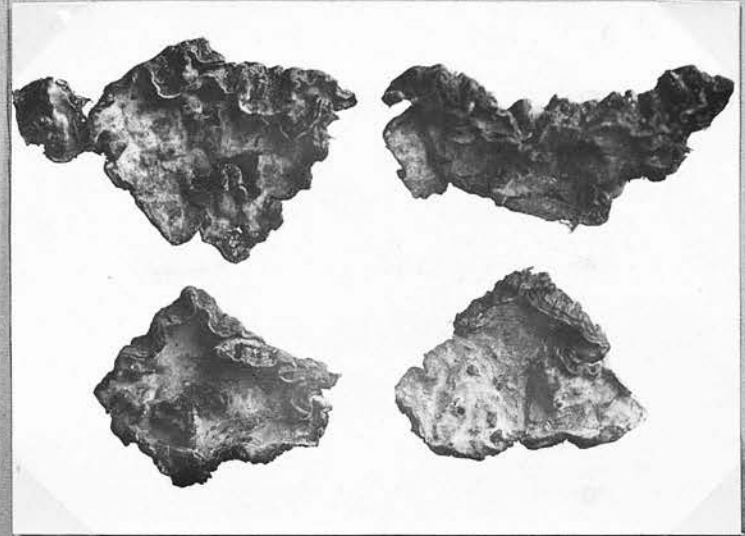




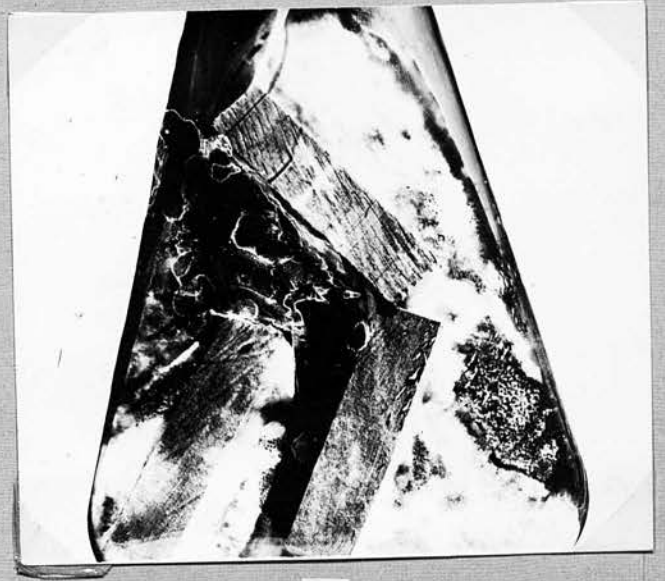
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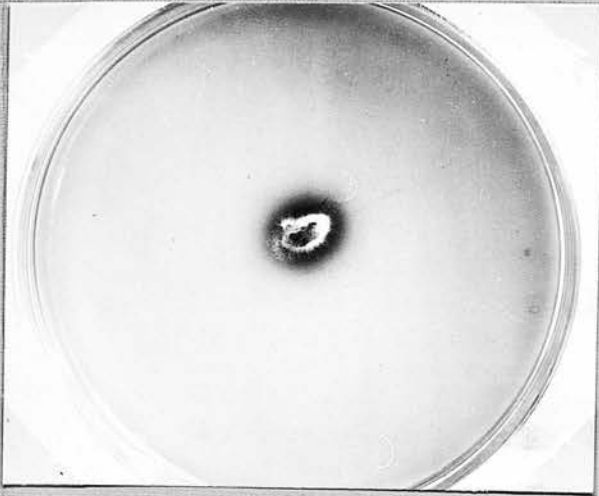
PLATE III.

Figs. 11 & 12. Stereum rugosum giving oxidase reactions after 3 and 7 days respectively on malt agar containing 0.5% gallic acid.

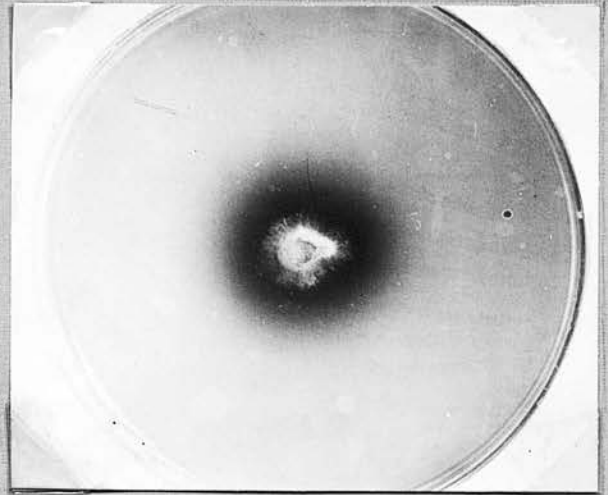
Figs. 13 & 14. Cultures of S. rugosum, 7 and 14-days-old, on malt agar at 23°C in darkness.

Fig. 15. Culture of S. rugosum, 21-days-old, on potato-dextrose agar at 23°C in darkness.

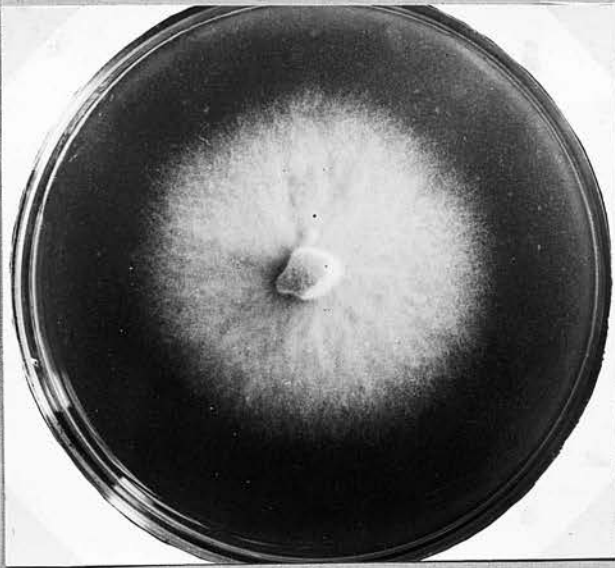
Fig. 16. Cultures of S. rugosum, 21-days-old, on potato-dextrose agar and malt agar in darkness and at 18°C (a, c) and 23°C (b, d) respectively.



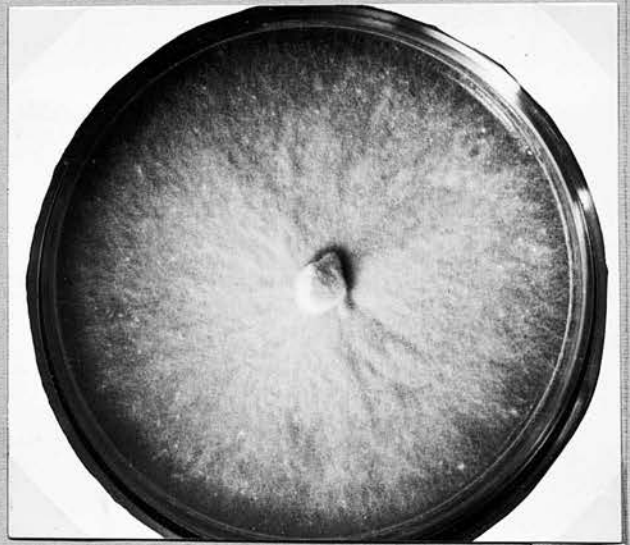
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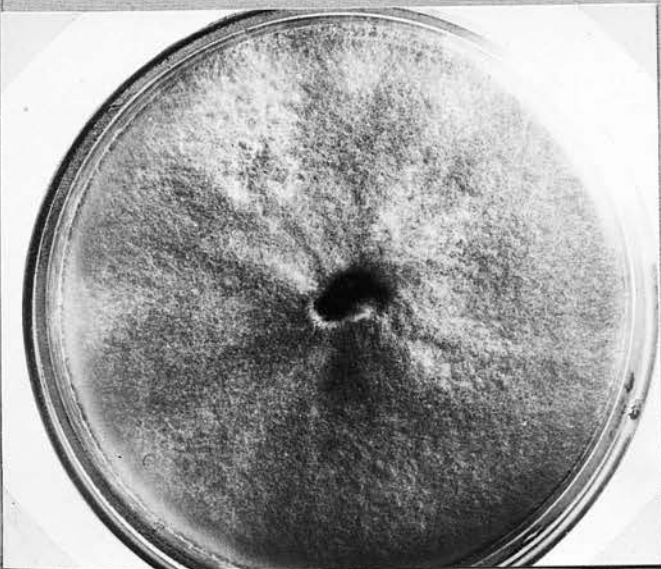
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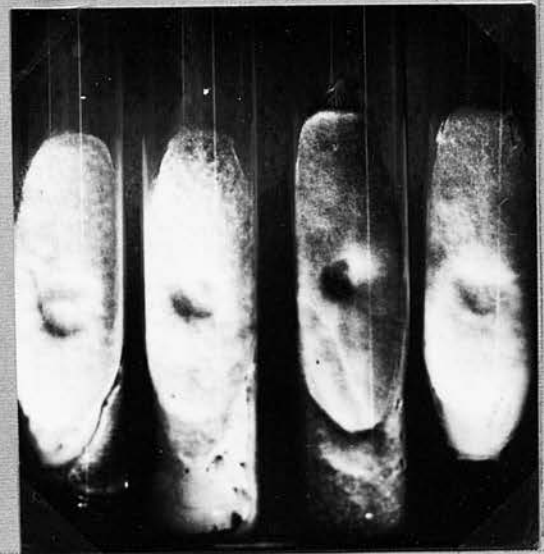
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a

b

c

d

16

PLATE IV.

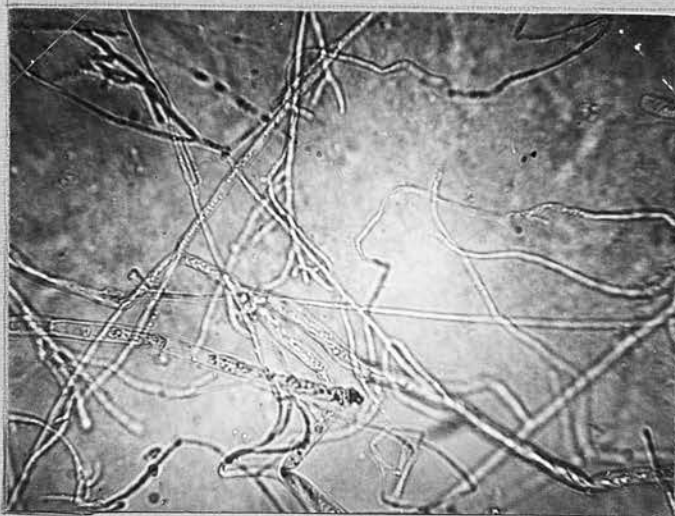
Fig. 17. Aerial mycelium of S. rugosum  
showing halicoid hyphae. (x 200).

Fig. 18. Submerged mycelium of S. rugosum.(x 200) .

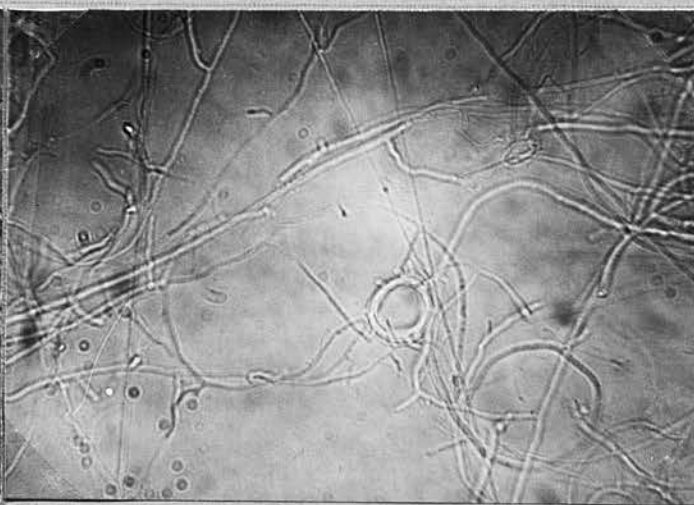
Fig. 19. Culture of S. rugosum on sapwood-  
blocks of oak for testing decay resistance  
in the laboratory.

Fig. 20. Culture of S. rugosum on heartwood-  
blocks of oak for testing decay resistance  
in the laboratory.

Fig. 21. Heartwood blocks of oak showing decay  
after 4 months' exposure to S. rugosum.



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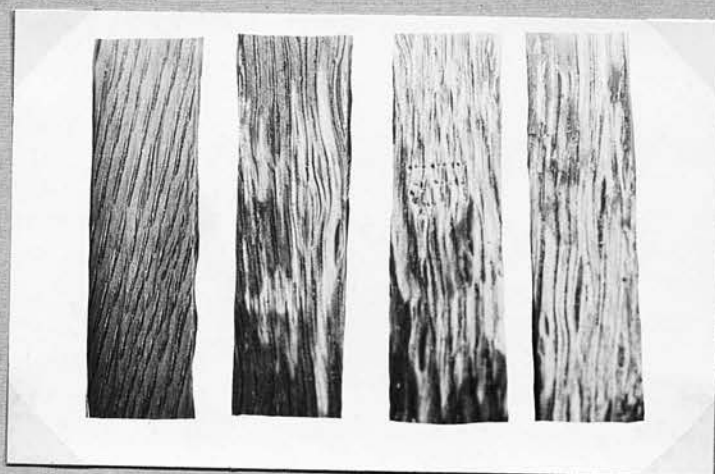
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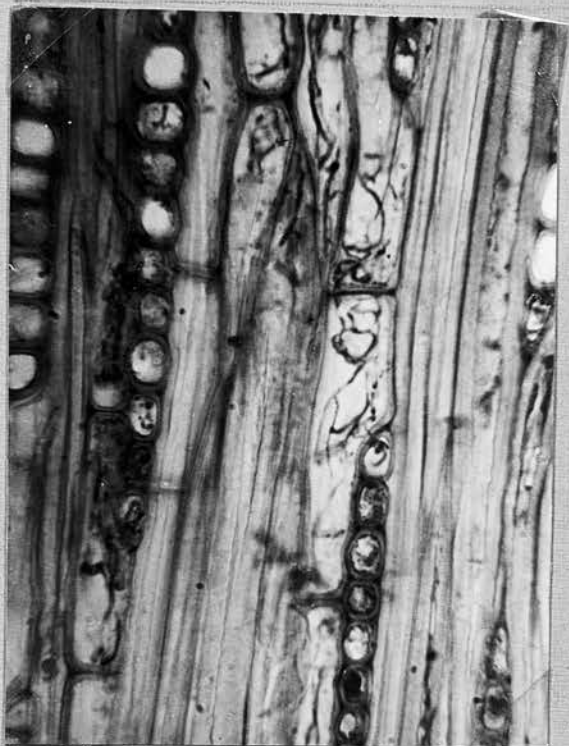
PLATE V.

Fig. 22. Part of a tangential longitudinal section through decayed heartwood of oak showing the presence of hyphae within fibres, wood parenchyma and ray cells. (x 100).

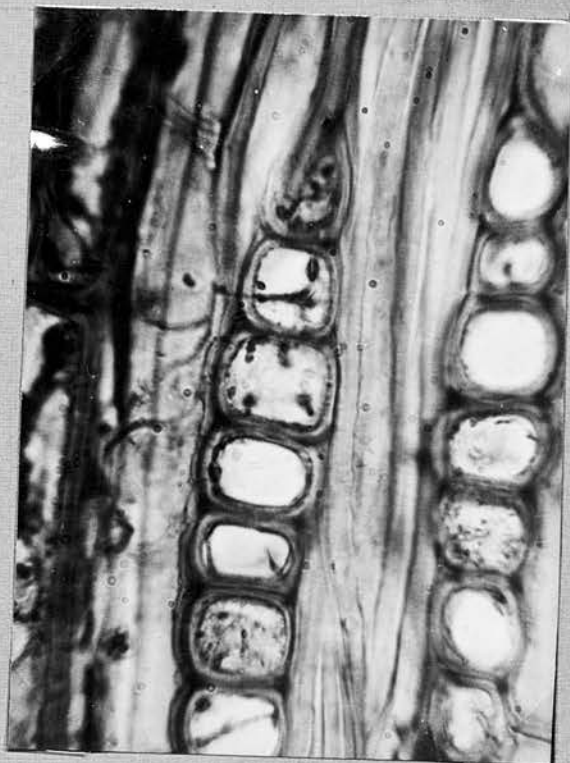
Fig. 23. Penetration of the walls of the parenchyma cells by the hyphae; the boreholes can be clearly recognised. (x 450).

Figs. 24 & 25. Transverse sections through heartwood of oak showing advanced stage of decay. (x 100).

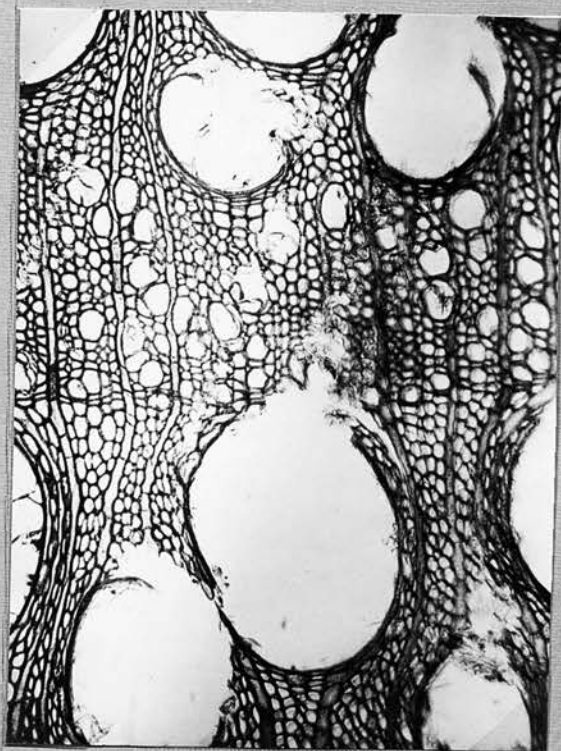
PLATE V



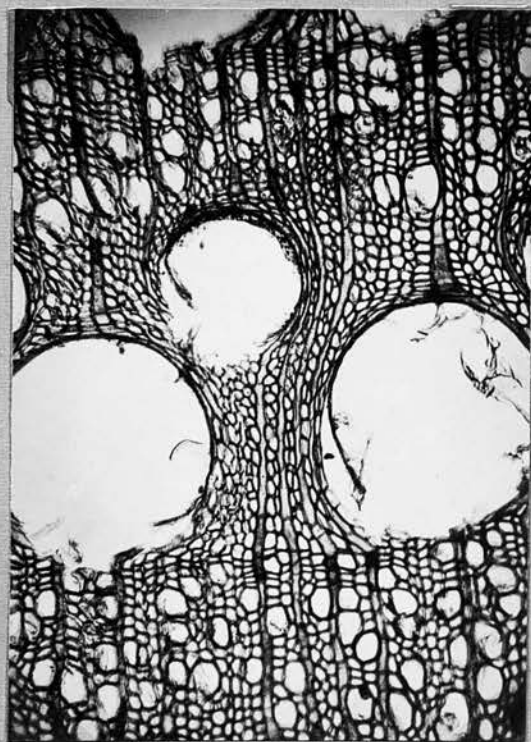
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PLATE VI.

Figs. 26 & 27. Inoculations on trunks  
of healthy oak showing infections  
after 4 months.

PLATE VI



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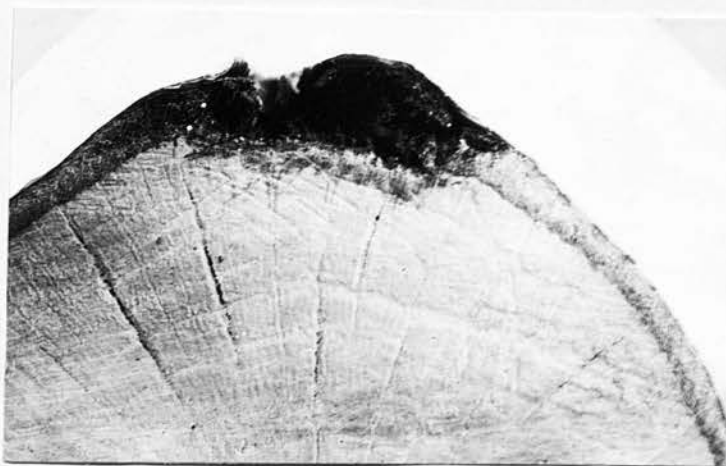
PLATE VII

Fig. 28.            Photograph showing infection on  
Oak, nine months after inoculation with  
Stereum rugosum.

Fig. 29.            Cross section of Oak through the  
same area of infection, showing the  
cavity and rot in the sapwood.



28



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